211 (b) Research Group

Via Certified Mail

FYI-0505-001423 MR 246741

NON-CONFIDENTIAL INFORMATION

May 25, 2005

Document Processing Center (Mail Code 7407M)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, DC 20460-0001

CONTAIN NO CBI

Re: TSCA 8(e) and FYI Supplemental Submission – Gasoline MTBE Vapor Condensate: Docket Nos. 8EHQ-102-15068 and FYI-0202-01423

Dear Sir/Madam:

The 211(b) Research Group is an unincorporated group of US fuel and fuel additive manufacturers affiliated by contractual obligation to meet the testing requirements of Section 211(b)(2) and 211(e) of the Clean Air Act. In 2002, FYI and TSCA 8(e) submissions were made based on the results of a developmental toxicity study on gasoline MTBE vapor condensate (see below). With the present filing, the 211(b) Research Group (RG), on behalf of its member companies, is submitting two supplemental draft reports from subsequent testing of the same material (i.e., gasoline MTBE vapor condensate).

On January 25, 2002, Equiva Services LLC (a member of the RG) provided EPA with preliminary results from a developmental toxicity study in female CD-1 mice exposed by inhalation (whole-body) to vapor derived from condensate of the 10% lowest-boiling fraction of a gasoline MTBE mixture. This information was provided to the Agency in accordance with provisions under TSCA Section 8(e). On January 30, 2002, ExxonMobil, another member of the RG, submitted an FYI on the same results. The findings included two types of unusual malformations, namely ectopia cordis and gastroschisis. These findings occurred at a very low incidence and were not observed in the highest exposure group.

In response to these findings, the RG sponsored a follow-up study that included a replicate of the first study design plus an additional treatment group at a higher exposure concentration. The dams in the additional treatment group were exposed to 30,000 mg/m^3

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for 6 hr/d on gestation days 5 through 10, rather than exposures on gestation days 5-16 for the other three treatment groups. A range-finding study was conducted to assure that the dams could tolerate 30,000 mg/m^3, which was 75% of the lower explosive limit of the test material.

In the range-finding study, neither ectopia cordis nor gastroschisis was observed.

In the full follow-up study, there were no findings of ectopia cordis or gastroschisis in animals treated similarly and at the same concentrations as those in the original study (2,000 mg/m^3; 10,000 mg/m^3; 20,000 mg/m^3). In the additional 30,000 mg/m^3 group, one fetus (out of 407 fetuses) in one litter (out of 33 litters) exhibited gastroschisis.

Details of the design, conduct, and results of the follow-up study and its companion range-finding study are provided in the enclosed copies of draft reports for both studies entitled:

- Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD® Mice
- Range-Finding Tolerance Study for the Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD® Mice

When the final reports for these studies are complete, they will be submitted to the EPA Office of Transportation and Air Quality, Transportation and Regional Programs Division, as part of the requirements of Clean Air Act Section 211(b)(2) and 211(e) (Docket No. A-90-07). If you require further information please contact me at 202-682-8344, or by mail at this address.

Regards,

Lorraine E. Twerdok

Lorraine Twerdok, Ph.D., DABT
Administrator, 211(b) Research Group

Enclosures (2): Draft Final Report - "*Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD® Mice*"
Draft Final Report - "*Range-Finding Tolerance Study for the Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD® Mice*"

cc: Monica Alvarez, EPA
Joe Sopata, EPA

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may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control number for EPA's regulations, after initial display in the final rule, are listed in 40 CFR part 9.

VI. References

1. U.S. EPA, OPPT. *I. Ethylene Dichloride (107–06–2).* Pp 24–27 *In*: "TSCA Section 4 Findings for 21 Hazardous Air Pollutants: A Supporting Document for Proposed Hazardous Air Pollutants (HAPs) Test Rule." (June 25, 1996).

2. The HAP Task Force. Letter from Peter E. Voytek to Charles M. Auer with attachment entitled: "Proposal for Pharmacokinetics Study of Ethylene Dichloride, November 22, 1996." (November 22, 1996).

3. U.S. EPA. Letter from Charles M. Auer to Peter E. Voytek with attachment entitled: "Preliminary EPA Technical Analysis of Proposed Industry Pharmacokinetics (PK) Strategy for Ethylene Dichloride, June, 1997." (June 26, 1997).

4. The HAP Task Force. Letter from Peter E. Voytek to Charles M. Auer, U.S. EPA. (March 19, 1999).

5. U.S. EPA. Letter from Charles M. Auer to Peter E. Voytek, HAP Task Force, Re: ECA Development of Ethylene Dichloride (EDC) (OPPTS 42197C, with attachment: "EDC ECA—DRAFT, dated February, 2001." (February 13, 2001).

6. Final Enforceable Consent Agreement for Ethylene Dichloride and Accompanying Testing Consent Order, signed by EPA on May 13, 2003.

7. D'Souza, R.W., Francis, W.R., Bruce R.D., and Andersen, M.E. *Physiologically based phamacokinetic model for ethylene dichloride and its application in risk assessment.* Pp 286–301, *In*: Pharmacokinetics in Risk Assessment. National Academy Press. Washington, D.C. (1987).

8. D'Souza, R.W., Francis, W.R., and Andersen, M.E. *Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposure.* Journal of Pharmacology and Experimental Therapeutics 245(2):563–568. 1988.

9. Daniel, F.B., Robinson, M., Olson, G.R., York, R.G., and Condie, L.W. *Ten and ninety-day toxicity studies of 1,2-dichloroethane in Sprague-Dawley rats.* Drug and Chemical Toxicology 17: 463–477. 1994.

10. Alumot, E., Nachtomi, E., Mandel, E., Holstein, P., Bondi, A., and Herzberg, M. *Tolerance and acceptable daily intake of chlorinated fumigants in the rat diet.* Food, Cosmetics and Toxicology 14: 105–110. (1976).

11. Rao, K.S., Murray, J.S., Deacon, M.M., John, J.A., Calhoun, L.L., and Young, J.T. *Teratogenicity and reproduction studies in animals inhaling ethylene dichloride.* Banbury Report 5: 149–166. (1980).

12. Lane, R.W., Riddle, B.L., and Borzelleca, J.F. *Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice.* Toxicology and Applied Pharmacology 63: 409–421. 1982.

13. Payan, J.P., Saillenfait, A.M., Bonnet, P., Fabry, J.P., Langonne, I., and Sabate J.P. *Assessment of the developmental toxicity and placental transfer of the 1,2-dichloroethane in rats.* Fundamental and Applied Toxicology 28: 187–198. 1995.

14. Sherwood, R.L., O'Shea, W., Thomas, P.T., Ratajczak, H.V., and Aranyi, C. *Effects of inhalation of ethylene dichloride on pulmonary defenses of mice and rats.* Toxicology and Applied Pharmacology 91: 491–496. 1987.

15. U.S. EPA, OPPTS. "Burden Estimates for the Enforceable Consent Agreement for Ethylene Dichloride." (January 31, 2002).

List of Subjects

Environmental protection, Hazardous chemicals.

Dated: May 13, 2003.

Stephen Johnson,

Assistant Administrator for Prevention, Pesticides and Toxic Substances.

[FR Doc. 03–13721 Filed 6–2–03; 8:45 am]

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ENVIRONMENTAL PROTECTION AGENCY

[OPPT–2002–0067; FRL–7287–4]

TSCA Section 8(e); Notification of Substantial Risk; Policy Clarification and Reporting Guidance

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: EPA is hereby finalizing revisions to certain parts of EPA's "Statement of Interpretation and Enforcement Policy; Notification of Substantial Risk" (policy statement) issued March 16, 1978, concerning the reporting of "substantial risk" information pursuant to section 8(e) of the Toxic Substances Control Act (TSCA). EPA is making these revisions after having considered public comments that were solicited in 1993 and 1995. Specifically, the revisions address the reporting of information on the release of chemical substances to, and the detection of chemical substances in, environmental media, the reporting deadline for written "substantial risk" information, and the circumstances under which certain information need not be reported to EPA under section 8(e) of TSCA. EPA is republishing the policy statement in its entirety in this document, including both those portions of the policy statement that are revised and those portions that are not affected by any revisions. Since the policy statement was published in 1978, this republication is intended to ensure that a single reference source for the TSCA section 8(e) policy and guidance is easily available to the regulated community and other interested parties.

FOR FURTHER INFORMATION CONTACT: *For general information contact*: Barbara Cunningham, Director, Environmental Assistance Division (7408M), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001; telephone number: (202) 554–1404; e-mail address: TSCA-Hotline@epa.gov.

For technical information contact: Richard Hefter, Chief, High Production Volume Chemicals Branch, Risk Assessment Division, Office Pollution Prevention and Toxics, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001; telephone number: (202) 564–7649; e-mail address: hefter.richard@epa.gov.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

You may be potentially affected by this action if you manufacture, process, import, or distribute in commerce chemical substances and mixtures. Potentially affected entities may include, but are not limited to:

- Chemical manufacturers, processors, and distributors (NAICS 325)
- Petroleum refiners and distributors (NAICS 324)
- Manufacturers of plastic parts and components (NAICS 325211)
- Paints and coatings and adhesive manufacturing (NAICS 3255)
- Cleaning compounds and similar products manufacturing (NAICS 3256)
- Electronics manufacturing (NAICS 334 and 335)
- Automobiles manufacturing (NAICS 3361)

• Aircraft manufacturing (NAICS 336411)

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in this unit could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether this action might apply to certain entities. To determine whether you or your business may be affected by this action, you should carefully examine the applicability provisions in Unit VIII., Part II., of this document. If you have any questions regarding the applicability of this action to a particular entity, consult the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

B. How Can I Get Copies of this Document and Other Related Information?

1. *Docket.* EPA has established an official public docket for this action under docket identification (ID) number OPPT–2002–0067. The official public docket consists of the documents specifically referenced in this action, any public comments received, and other information related to this action. Although a part of the official docket, the public docket does not include Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. The official public docket is the collection of materials that is available for public viewing at the EPA Docket Center, Rm. B102–Reading Room, EPA West, 1301 Constitution Ave., NW., Washington, DC. The EPA Docket Center is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The EPA Docket Center Reading Room telephone number is (202) 566–1744 and the telephone number for the OPPT Docket, which is located in EPA Docket Center, is (202) 566–0280.

2. *Electronic access.* You may access this **Federal Register** document electronically through the EPA Internet under the "**Federal Register**" listings at http://www.epa.gov/fedrgstr/. Information about the Office of Prevention, Pesticides and Toxic Substances (OPPTS) and OPPTS-related programs is available from http://www.epa.gov/opptsmnt/.

An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at http://www.epa.gov/edocket/ to submit or view public comments, access the index listing of the contents of the official public docket, and to access those documents in the public docket that are available electronically. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in Unit I.B.1. Once in the system, select "search," then key in the appropriate docket ID number.

II. Background

A. What Action is the Agency Taking?

The Agency is revising and clarifying certain provisions of the TSCA section 8(e) policy statement issued in 1978. Specifically the Agency is changing the interpretation that section 8(e) notices should be submitted within 15 working days by lengthening the reporting period to 30 calendar days. The Agency is revising and clarifying the guidance regarding the release and detection of chemical substances in environmental media, which includes previously unsuspected chemical contamination such as in soil and ground water, and emergency incidents of environmental contamination such as spills to water and releases to the atmosphere. Also, the Agency is expanding the types of information that it believes need not be reported under section 8(e) and changing the reporting periods to provide additional time for industry compliance with TSCA section 8(e). In addition, EPA is updating certain reporting contact phone numbers and the address for reporting section 8(e) notices.

While the Agency is only revising portions of the 1978 guidance it has issued in earlier documents, EPA is including in this **Federal Register** document, along with the revised guidance, those portions of earlier guidance documents that are not being changed. In that way, members of the regulated community will be able to find all current EPA guidance on compliance with section 8(e) in this **Federal Register** document, without having to consult older documents as well.

The Agency is including in this guidance document its preferences for how and where section 8(e) notices should be submitted. Although these preferences could be codified in procedural rules under the Administrative Procedures Act (APA), 5 U.S.C. 551 *et seq.*, EPA is not at this time adopting them as rules. While submitters of section 8(e) notices are not therefore obligated to comply with the preferences articulated in this document, EPA encourages submitters to consider and follow them when preparing and submitting TSCA section 8(e) notices.

Finally, the bulk of this document contains EPA's guidance on certain types of information it currently believes generally meet the statutory standard of "information which reasonably supports the conclusion that such substance or mixture presents a substantial risk of injury to health or the environment." Some of this guidance is new, and reflects public comment following the Agency's requests for comments in 1993 and 1995. As noted earlier, this document also contains earlier guidance issued on section 8(e) that has not been changed and that is being reprinted here for the convenience of all interested persons.

During the Compliance Audit Program (CAP) (see Unit II.C.), EPA reviewed the provisions in the reporting guidance for incidents involving chemical contamination of the environment. The changes set out in this document were developed as a result of that review. In 1993, EPA issued a **Federal Register** notice (58 FR 37735, July 13, 1993) that proposed changes to the reporting guidance. In 1995, after consideration of comments received on the 1993 proposal, EPA sought additional public comment on proposed changes to the reporting guidance (60 FR 14756, March 20, 1995) (FRL–4937–6). Unit III. describes the changes EPA proposed, the comments received on the proposed changes, and the Agency's resolution of the issues raised by the comments.

B. What is the Agency's Authority for Taking this Action?

TSCA section 8(e) states, "Any person who manufactures, [imports,] processes, or distributes in commerce a chemical substance or mixture and who obtains information which reasonably supports the conclusion that such substance or mixture presents a substantial risk of injury to health or the environment shall immediately inform the [EPA] Administrator of such information unless such person has actual knowledge that the Administrator has been adequately informed of such information." 15 U.S.C. 2607(e).

EPA hopes and expects that this guidance will be useful to manufacturers, including importers, processors, and distributers of chemical substances in fulfilling their responsibilities under section 8(e). This guidance is not, however, a substitute for rulemaking and it does not impose any binding requirements upon either the regulated community or the Agency. In any particular set of circumstances, any person who has a question about

the applicability of section 8(e) to certain information is welcome to contact EPA. In responding to such person, the Agency will consider the guidance contained in this document, but the guidance will not be determinative. It is also important to point out that the guidance provided will not be unalterable, and that the Agency may revise this guidance without notice or an opportunity to comment. EPA has sought public comment on this guidance so that it can ensure the utility of the guidance for the intended audience. If it becomes necessary, the Agency will revise this guidance.

C. What is the Agency's Current Policy on and Interpretation of the TSCA Section 8(e) Reporting Requirements?

The section 8(e) reporting requirements became effective on January 1, 1977, the effective date of TSCA. The statutory language of section 8(e) requires the exercise of a certain degree of judgment in determining what information must be reported. Although section 8(e) is self-implementing, EPA issued a proposed policy statement in the **Federal Register** of September 9, 1977 (42 FR 45362), and sought public comment with regard to the Agency's interpretation and implementation of section 8(e). Following receipt and consideration of public comments, on March 16, 1978 (43 FR 11110) (FRL–849–2), EPA issued a final TSCA section 8(e) policy statement hereinafter cited as the "1978 Policy Statement." The 1978 Policy Statement described the types of information that EPA considers reportable under section 8(e) and described the procedures for reporting such information to EPA.

In the **Federal Register** of February 1, 1991 (56 FR 4128), the Agency announced a one-time voluntary TSCA section 8(e) CAP. The CAP was designed primarily to: (1) Obtain any section 8(e) information that was required to have been submitted to EPA before the CAP, and (2) encourage companies to voluntarily search ("audit") their files for data reportable under section 8(e). The TSCA section 8(e) CAP established a schedule of monetary penalties for failure to submit section 8(e) data before the CAP, and also established a ceiling on penalties that would be collected from any single company.

D. The Reason for Issuing Revised Guidance

Companies considering whether to participate in the CAP had raised questions about Parts V.(b)(1) and V.(c) of the 1978 Policy Statement. Those sections outlined the reportability of data on "widespread and previously unsuspected distribution in environmental media" and "emergency incidents of environmental contamination," respectively. In order to answer the questions raised by the companies, the Agency reviewed existing section 8(e) guidance and determined that Parts V.(b)(1) and V.(c) of the 1978 Policy Statement needed clarification and refinement. Therefore, in the **Federal Register** of June 20, 1991 (56 FR 28458), EPA announced that the Agency was suspending application of Parts V.(b)(1) and V.(c) of the 1978 Policy Statement.

That **Federal Register** document also stated that EPA was going to provide more specific guidance about the types of information on environmental releases and detection of environmental contamination that should be submitted under section 8(e). Phase 2 of the CAP, which was to deal with data on environmental contamination, would be triggered by publication of that revised guidance (phase 1 of the CAP had dealt with studies of "effects" of toxic substances on health or the environment.). On July 13, 1993, EPA issued a **Federal Register** document (58 FR 37735) that proposed changes to the 1978 Policy Statement, clarifying the types of environmental contamination data that EPA believes are subject to section 8(e) reporting.

Comments received on the proposed changes took issue with a number of the revisions proposed by the Agency as well as with the original guidance. Based on the comments received, it became apparent that any final guidance would likely be significantly different from previous guidance and should therefore be applied prospectively. Since the CAP was essentially a retrospective exercise, the decision to make substantial revisions in the guidance for reporting on environmental contamination called into question the utility of carrying out phase 2. Consequently, the Agency, in consultation with CAP participants, decided to conclude the CAP after phase 1 "effects" reporting. Letters were sent to CAP participants announcing the change in the program, and the CAP was terminated on May 15, 1996. EPA reached final settlements with CAP participants, announced those settlements on October 15, 1996, and collected payment for stipulated penalties.

III. Section 8(e) Policy Clarifications and Revisions

EPA's interpretation of section 8(e) is that it requires the reporting of certain "substantial risk" information concerning the release of chemical substances to, and the detection of chemical substances in, any environmental medium. In order to enhance implementation of TSCA section 8(e), EPA is, in this **Federal Register** document, publishing a complete version of the policy statement which reflects comments received on proposed refinements to the policy statement published on July 13, 1993 (58 FR 37735), and March 20, 1995 (60 FR 14756). EPA has also decided to reinstate application of Part V.(c) relating to "emergency incidents of environmental contamination," which was suspended on June 20, 1991 (56 FR 28458).

A. What Changes were Proposed in 1993?

In a notice published in the **Federal Register** on July 13, 1993 (58 FR 37735), EPA proposed the following changes to the 1978 Policy Statement:

1. Revise the 1978 reporting guidance as to when the discovery of "widespread and previously unsuspected [chemical] distribution in environmental media" would trigger a substantial risk notice under section 8(e). EPA indicated that the key elements to consider would be the known hazard potential of the contaminant, how "widespread" the substance is in the environment, and the potential for actual human or environmental exposure. EPA further stated that the weight to be given exposure considerations would be judged in light of hazard potential, i.e., the more hazardous the chemical the less one would weigh exposure considerations.

2. Expand the categories of information cited in the 1978 reporting guidance that EPA believed no longer need to be reported to under section 8(e). The major change proposed was intended to reduce the potential for TSCA section 8(e) submissions to be duplicative of reporting under other mandates, by allowing an exemption for information reported under other EPA reporting requirements (including those delegated to the states). Also, a clarification of what would constitute "corroborative" data not subject to reporting was proposed.

3. Change the interpretation that section 8(e) notices for information other than "emergency incidents of environmental contamination" should be submitted within 15 working days by lengthening the reporting period to 30 calendar days.

4. Eliminate the need to follow up an emergency release notification under Part V.(c) with a written report.

5. Clarify standards for claiming CBI in section 8(e) notices.

6. Correct the address under Part IX. of the 1978 Policy Statement.

B. Summary of Public Comments on the 1993 and 1995 Proposed Revisions and EPA's Responses

In addition to the brief summaries of public comments and Agency responses presented in this **Federal Register** document, EPA has prepared a "response to comments" document that addresses in greater detail the significant comments it received on the proposed changes. The public version of the "response to comments" document, which does not contain any CBI information, is publicly available in the docket described in Unit I.B.1 of this document.

1. *Comments on the 1993 proposed changes.* EPA received comments from 49 companies and industry associations in response to the 1993 **Federal Register** document. Commenters suggested that EPA's proposed plan for environmental reporting lacked criteria that were sufficiently clear to enable companies to separate "routine" releases, which need not be reported, from the "extraordinary" releases, which were to be reported under section 8(e). Commenters stated that EPA should provide clearer criteria for determining when a situation presents a "substantial risk," and should provide as many "bright lines" as possible to indicate what would and would not be reportable under section 8(e). Specifically, commenters:

- Questioned EPA's interpretation of when contamination would be "widespread."
- Stated that only a contaminant's "known" toxicity should be considered.
- Stated that for contamination to be reportable, it must be "previously unsuspected" contamination.
- Stated that the contamination must result in actual or high probability of significant exposure to humans or non-human organisms.
- Stated that any contamination to be reported under section 8(e) must "present" a substantial risk rather than only a speculative "may present."
- Proposed that EPA establish a decision tree that companies could follow to determine whether to report incidents involving environmental contamination under section 8(e). Commenters stated that if companies had sequential criteria, they would be in a much better position to comply with the reporting requirements of section 8(e).
- Supported the change to the section 8(e) notice reporting period from 15 working days to 30 calendar days.

The bulk of the remaining comments concerned circumstances under which companies need not report information to EPA. EPA had proposed to exempt from reporting under TSCA section 8(e) information companies were required to report under other EPA authorities (including those delegated to the States). However, the exemption would only apply if the information was submitted under the other authorities within 30 days of obtaining the information. Commenters believed that this would offer little relief because many of the other authorities have reporting periods longer than 30 days. The companies would either have to accelerate their reporting under authorities other than TSCA section 8(e) or submit two reports, one within 30 days under section 8(e) and another within the time frame of the other requirement. The commenters suggested allowing a longer time frame, i.e., 90 days or longer, for that information submitted under authorities other than TSCA section 8(e).

Commenters also suggested expanding the "other authorities" exemption to include reporting under all Federal environmental statutes as well as State laws and regulations, especially when a site is undergoing remediation for contamination with hazardous waste and any environmental or health threats associated with those contaminants are being addressed in the course of the remediation.

Finally, EPA received comments on the relationship of the revised guidance to phase 2 of the CAP. The sentiment expressed by all those who commented on this issue was that, given the limited guidance in the 1978 Policy Statement, EPA's suspension of the guidance section on environmental contamination, and the likelihood that EPA's final guidance would be essentially "new," the final guidance should only be enforced prospectively. Consequently, companies should not be subject to any liability for past failures to report under the criteria of the "new" guidance.

2. *EPA's response to comments on the 1993 proposed changes; the 1995 proposed draft guidance.* In response to the comments received on the 1993 proposed changes to the 1978 guidance, on March 20, 1995, EPA issued revised proposed guidance to address the commenters' concerns.

First, in the 1995 notice, EPA proposed clarifications to the situations involving environmental contamination which EPA believes would need to be reported. Language suggested in comments to the 1993 notice was adopted, specifying that the contamination must be "previously unsuspected," that "exposure" has occurred or there is a substantial likelihood that it will occur, and that the chemical(s) in question is "known" to cause serious adverse effects. EPA stated that information on those effects could be obtained from several sources:

- Databases available to the public (online or in paper versions), such as the National Library of Medicine (NLM) databases (Toxline, Medline, Hazardous Substances Data Bank, etc.), National Institute for Occupational Safety and Health (NIOSH) Registry of Toxic Effects of Chemical Substances (RTECS), EPA's Aquatic Toxicity Information Retrieval database (AQUIRE) (Now the Ecotoxicology (ECOTOX) database) www.epa.gov/ecotox/.
- Reports to EPA or other government agencies.
- Unpublished data known to the person or entity subject to reporting.

As regards the issue of what is meant by "known" to cause serious adverse effects, EPA did not mean that the effects must be conclusively shown and did not intend a higher standard of certainty than for the "effects" reporting part of the 1978 Policy Statement. In that notice, EPA stated that all that is needed for an effect to be "known" is that the information reasonably supports that the chemical can cause the effect(s) of concern. This issue is addressed in the 1978 Policy Statement in EPA's response to comments that questioned the use of "may suggest" language regarding information obtained and the reporting of substantial risk information (see Supplementary Information paragraph (3) of the 1978 Policy Statement).

In addition, EPA agreed to allow the use of "benchmark levels" to help determine if the information should be reported. EPA has established benchmark levels for various substances. Benchmark levels are concentrations that either trigger a regulatory response, or concentrations above which a substance is presumed to present a risk to health and/or the environment. For instance, the Agency has developed Reference Doses (RfD's) for numerous substances under its Integrated Risk Information System (IRIS). Reference doses establish a level of exposure where no adverse effects would be expected to be manifested. Thus, if a person found groundwater contaminated with a chemical at a level that did not exceed the RfD for that

substance, the person could assume that a substantial risk does not exist. It should be noted that benchmark levels are often medium-specific, so their use should be limited accordingly. Examples of certain benchmark levels can be found at the following EPA Web sites: http://www.epa.gov/iris/ and http://www.epa.gov/ost/drinking/standards/dwstandards.pdf.

Second, EPA increased the number of types of information that it believed need not be reported under TSCA section 8(e). The types of information proposed to be exempted included:

- Draft and final reports made available to the public by other Federal agencies.
- Data obtained from scientific journals and databases, including, but not limited to, those to which EPA subscribes.
- Information obtained from news publications and radio/television broadcasts.
- Information obtained at scientific meetings or conferences where EPA is the sponsor, where the information is presented by an EPA employee or contractor acting on behalf of EPA, and at other similar meetings, provided that such information is cited or abstracted in a scientific journal or database within 90 days of a person subject to reporting under section 8(e) obtaining such information.

The rationale for these proposed changes was to relieve persons who are potentially subject to reporting under section 8(e) from the burden of considering information from secondary sources when the secondary source does not provide sufficient information for a person to judge whether the information should be reported. For instance, a manufacturer of a chemical might obtain a news article about research done by another company. A person reading the article would need the underlying study to evaluate the true significance of the results of the research and, based on that evaluation, make a judgment as to whether there is a substantial risk of injury to human health or the environment. In such a case, the potential reporting obligation falls on the company that generated the research discussed in the news article.

Third, EPA retained the interpretation proposed in the 1993 **Federal Register** notice that section 8(e) notices for information other than "emergency incidents of environmental contamination," should be submitted within 30 calendar days. EPA continues to believe that the change from 15 working days to 30 calendar days would significantly relieve the burden on persons subject to section 8(e) reporting without substantially affecting EPA's ability to appropriately evaluate and respond in a timely manner to the reported information.

Fourth, EPA identified the group of statutes for which exemptions would be granted from reporting of non-emergency information under TSCA section 8(e), specifying the other statutes administered by EPA and those for which implementation was delegated to the States. The maximum allowable reporting period, in lieu of reporting under section 8(e), under those other authorities was increased from 30 to 90 days from the date reportable non-emergency situations of chemical contamination was obtained by a person subject to section 8(e), i.e, persons reporting to the other authorities within the 90–day time frame would be exempt from reporting the information under section 8(e). EPA believed that extending the time for reporting non-emergency situations of chemical contamination would allow for those instances where assembling several types of information in order to determine whether section 8(e) applies could take more than 30 days and was consistent with the majority of the reporting periods under the other statutes.

Fifth, if the Federal government or a State requires that information be submitted on a site remediation program carried out under Federal or State regulations, that information would not have to be separately submitted under section 8(e) beyond an initial section 8(e) notification. The Agency believed that once the chemical contamination situation has been identified, such as by a notice under section 8(e), and the site is undergoing remediation, little if any additional benefit is gained by subsequent section 8(e) reporting concerning that chemical contamination situation at the same site.

Sixth, usually only the person who operates or owns a site at which environmental contamination has occurred would have the responsibility to report under section 8(e). It is unlikely that a person not associated with a site as an owner or operator would have access to a sufficiently wide range of information about an environmental contamination situation to determine whether data on the contamination meet the test for section 8(e) reporting. This is unlike the acquisition of effects test data, because data on effects are not site-specific and have general applicability for production and use of the chemical of interest in the United States. Similarly, persons subject to section 8(e) would not have to report information obtained about a site outside the United States unless there is potential for contamination from that site to enter the United States.

Seventh, because of the number of changes made to the proposed guidance in the 1995 **Federal Register** notice and the fact that it represented a significant change from the original guidance suspended on June 20, 1991, the Agency concluded that the revised guidance when issued should be applied prospectively. This eliminates the need for companies to review files currently in their possession for information that may be subject to section 8(e) reporting in accordance with the revised guidance. However, data in such files could be subject to section 8(e) reporting if data obtained by a company after issuance of the revised guidance triggered a review of such preexisting data and in doing so the combination of preexisting and new data met the section 8(e) reporting criteria.

Eighth, the Agency stated that it would develop, in cooperation with interested parties, a "question and answer" (Q. and A.) document that would provide further detail and "real world" examples to further assist persons in fulfilling their section 8(e) reporting responsibilities as regards the revised guidance. The Agency stated that it intends to work with interested parties to prepare such a Q. and A. document, which EPA expects to have available several months from the issuance of the final reporting guidance. At that time, the Agency intends to post the Q. and A. document on the TSCA section 8(e) homepage (http://www.epa.gov/oppt/tsca8e). A copy may also be obtained from the contacts listed under **FOR FURTHER INFORMATION CONTACT**. As additional examples, or questions and answers are identified as being of potential value to share broadly, the Agency will refine this Q. and A. document.

Finally, some commenters requested an additional opportunity to review the revised draft guidance developed in response to the extensive comments of the proposed revisions in the July 13, 1993 **Federal Register** notice. On March 20, 1995 (58 FR 37735), the Agency published a notice of availability in the **Federal Register** of the revised draft guidance and allowed 45 days for comment. The 1995 draft guidance substantially responded to the comments received on the 1993 proposed revisions.

3. *Comments on the 1995 proposed changes and EPA's response.* In response to the Agency's request for comment on the revised draft guidance published in 1995, EPA received

comments from 22 companies and trade associations. The commenters generally agreed that the changes made by EPA addressed most of their major comments on the 1993 proposed guidance, and that the 1995 revised guidance was a significant improvement. For example, the Monsanto Company stated: "The reproposed guidance, as summarized in the draft policy text for public comment dated March 9, 1995, is a significant improvement over the guidance published July 13, 1993. The reproposed guidance significantly minimizes the duplicative over-reporting burden that characterized the earlier guidance document. We support the reproposed guidance document and believe it is generally consistent with the Congressional intent of the original drafters of TSCA, as well as current Agency and Congressional efforts to reform government reporting requirements to minimize duplicative and unneeded over-reporting. The reproposed guidance document on environmental release/contamination is a significant move in the direction of clarifying the Agency's need for information that reasonably supports a conclusion of substantial risk." (Ref. 1).

In addition to their statements of support for the proposed changes, the commenters requested a number of clarifications/definitions of terms, editorial rewordings, and other less substantive changes that are addressed in a "response-to-comments" document that can be found in the docket as described in Unit I.B.1. Commenters expressed strong support for making the new guidance prospective, ending the CAP at phase 1, and developing a Q. and A. document. As previously discussed, EPA is in agreement with those comments.

One major area where industry commenters requested further changes was the exemption from reporting under section 8(e) for data submitted to EPA or other agencies under other authorities. The commenters were concerned about the extent to which exemptions from reporting under section 8(e) would be granted for reporting under authorities other than EPA statutes administered either by the Agency or, where implementation of an EPA statute has been delegated to the States. EPA had proposed to reduce the potential for duplicative submission under TSCA section 8(e) authorities by allowing an exemption to reporting under section 8(e) for all information which is required to be reported under other EPA statutes including where implementation had been delegated to the States, and where such reporting was required to be submitted within 90 days of being obtained. Industry commenters also questioned the length of the time period for reporting proposed by EPA. Industry commenters requested that the exemption be expanded to: (1) Include any mandatory reporting requirement whether Federal, State, or local, and (2) allow reporting within the time frame provided by the individual reporting authorities.

Regarding expanding the section 8(e) policy statement list of reporting authorities that would fall under a reporting exemption in Part VII. of the policy statement, the July 1993 and March 1995 proposals included an exemption to reporting only if the information was to be submitted under EPA statutes, including statutes such as the Clean Air Act, where implementation has been delegated in large part to the States. Delegation of implementation allowed a clear "nexus" to be shown between a State reporting requirement and EPA, thus following the statutory language of section 8(e) which does not require reporting if a company has "actual knowledge that the Administrator has been adequately informed of such information." The commenters would have EPA expand the reporting exemption by including any Federal, State, or local reporting requirements.

The issue of expanding the reporting authorities is problematic because of the statutory language in section 8(e). However, it is also relevant to look to the purpose of TSCA, and section 8(e) in particular, in light of the legislative history concerning how TSCA should be implemented. TSCA was designed to fill a number of regulatory gaps. Those included review of "new" chemicals, collection of test data on new and existing chemicals, and regulation of chemicals to address risks associated with chemicals' production, use, or disposal. Specifically, regarding the submission of test data, Congress wanted to avoid the potential for industry to withhold "information which would have revealed hazards associated with these chemicals at a much earlier date" (Ref. 2). Thus, the reporting requirement of section 8(e) of TSCA was established so that the Agency would be "adequately informed" to enable it to take corrective action if necessary. While Congress envisioned TSCA as filling a major gap in the regulatory framework protecting human health and the environment, it also directed the Administrator to avoid duplicating existing (and future) regulatory and enforcement authorities.

Given the statutory language of section 8(e), it is hard to make a case that the Administrator is adequately informed of reporting under State or local authorities, other than those reporting requirements that originate in laws administrated by EPA in which the United States Congress has provided for delegation to the States, and such delegation has occurred. Except where such delegation of EPA authority has occurred, the Agency believes reporting to a state government may not result in EPA getting important information in a timely manner and, therefore, EPA does not believe it is appropriate to exempt from section 8(e), information that is reported to state governments.

However, at least some information reported under other Federal authorities could be viewed differently. While there is not a direct statutory "nexus," often there is a considerable amount of interagency cooperation in dealing with environmental contamination situations, e.g., the National Response Center. To the extent EPA Headquarters and the Regions become involved in joint cleanups, assessments, etc., or act in advisory roles with other Federal agencies, the Administrator could reasonably be considered to be adequately informed. The Agency believes that information reported under other Federal authorities for site-specific contamination within 90 calendar days or immediately pursuant to a mandatory reporting requirement qualifies for exemption from section 8(e) reporting.

While this approach reduces the role of section 8(e) in the reporting of site-specific release/contamination information, Congress' goal in passing TSCA to ensure that important health and environmental related information are reported in a timely fashion will still be met. Further, since there is now a considerable array of Federal health and environmental reporting requirements, including section 8(e), which provide such information and for which there is enhanced public access, Congress's goal is not considered to be compromised by some of the expanded exemptions.

However, product contamination information that could be required to be submitted to the Consumer Product Safety Commission (CPSC) under their regulations is not analogous. CPSC has a more narrow purview (i.e., consumer product safety) and could not adequately assess or address chemical contamination from a product that may also have industrial/commercial applications or may present potential environmental risks during its manufacture and processing. In such instances, reporting to EPA, as well as CPSC would allow EPA, consistent with the intent of TSCA, to address all the potential risks presented, where appropriate. Consequently, EPA has

concluded that section 8(e) reporting will continue to be required for chemical product contamination, because EPA, uniquely among Federal agencies, has the authority to address all potential health and environmental risk aspects of a chemical's life cycle.

Regarding the issue of expanding the reporting exemption in Part VII. of the section 8 policy statement to allow reporting within the time frame provided by the individual reporting authorities, as originally proposed in 1993, companies would not be required to report information under section 8(e) if the information was required to be submitted under other EPA or EPA-delegated authorities, so long as the other statute required reporting within 30 days from the day a person who was required to report obtained information required to be submitted. Commenters noted that only a few of the regulations required reporting within 30 days, so the exemption would be of limited value given that companies would still be required to report the information under section 8(e) as well as under the other regulations. To address this concern, the reporting policy is being changed. Companies would be exempt from reporting information under section 8(e) as long as the company complies with the relevant reporting requirement of another statute, as described in Part VII. of the TSCA section 8(e) policy and guidance, that requires reporting within 90 days from the day a person obtained information required to be submitted. This change was based on information submitted by industry showing that roughly 70 percent of the reporting requirements have reporting periods of 90 days or less (see Ref. 3 at page 29, Table 1). Further, an examination of the cited reporting requirements shows that the 90–day period will capture reports that otherwise would be required under section 8(e), namely newly found environmental contamination from spills, leaking tanks, and other types of releases. By and large, the types of reporting for which the statutory time limits for filing of mandatory reports are longer than 90 days include periodic summary reports, minor operating changes allowed by permits, etc.

It appears that most or all of the exposure-related or site-specific release/detection information that might be considered reportable under section 8(e) would be required to be reported under other authorities within 90 days of such information being obtained. Therefore, there would be a negligible reduction of the reporting burden if authorities whose reporting time limits exceed 90 days were also exempted from reporting under section 8(e). Also, such a change seems inconsistent with the statutory language that substantial risk information be "immediately" reported. Given that a 90–day limit appears to resolve most of the problem with potentially duplicative reporting, and that longer limits may not be consistent with the statutory directive for "immediate reporting," EPA has decided to keep the reporting time limit at 90 days as proposed in the 1995 draft guidance.

Additionally, as proposed in the 1993 and reproposed 1995 draft guidance, EPA is adopting the interpretation that section 8(e) notices for information other than "emergency incidents of environmental contamination" should be submitted within 30 calendar days. Thus the Agency is changing in this guidance document its interpretation of the term "immediately" in this context. EPA believes the term should be interpreted more flexibly based upon the Agency's experience of processing and use of data reported under section 8(e) and comments received from interested parties. EPA has concluded that, with the exception of reporting related to emergency incidents of environmental contamination, section 8(e) reports should be submitted to EPA within 30 calendar days of obtaining the reportable information, instead of the 15 working days that was articulated in previous guidance. The Agency believes that application of this interpretation for the statutory term "immediately" will not adversely impact section 8(e)'s purpose of assuring that the Agency becomes aware of important risk-related information in a timely manner. In addition, providing 30 calendar days for reporting to the Agency is consistent with the regulations under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, which provides that agencies should not require a written response in fewer than 30 days after receipt without demonstrating that it is necessary to satisfy a statutory requirement or other substantial need (5 CFR 1320.5(d)(2)(ii)). Although TSCA section 8(e) clearly provides the necessary statutory justification to require a shorter response time, the Agency is using the minimum time frame established under the PRA to respond to the commenters who indicated the need for additional time to process a submission.

C. EPA's reinstatement of Part V.(c)

"*Emergency incidents of environmental contamination.*" Part V.(c) of the 1978 Policy Statement, which addresses what constitutes a "substantial risk" in the context of emergency incidents of environmental contamination, was suspended on June 20, 1991 (56 FR 28458). EPA has decided, for the following reasons, to reinstate Part V.(c):

- EPA is making a number of changes to the reporting guidance that would affect emergency incident reporting. Changes include reporting to the National Response Center, elimination of follow-up written section 8(e) reports, and expansion of the list of authorities persons could report under in lieu of section 8(e).
- Part V.(c) includes the basic elements of the new Part V.(b)(1) guidance: The adverse effect(s) in question have been ascribed to the chemical; human or environmental exposure may occur; exposure (in this case, an emergency release) threatens humans and/or non-human organisms with serious adverse effects.
- EPA believes such reporting under section 8(e) is still necessary. Although many release incidents are covered under other statutes, there may be instances where chemicals that have not yet been reviewed for release reporting under other EPA programs have the requisite hazard characteristics to require a response/notification if there is a release to the environment. In this regard, EPA agrees with a comment from the Chemical Manufacturers Association (CMA—CMA is now the American Chemistry Council) indicating that, if EPA retains the distinction between emergency and non-emergency situations of environmental contamination, "emergency" should be defined. CMA stated: "CMA believes an 'emergency' should be defined as a situation in which a significant threat to human health or the environment is imminent or already present, and where immediate action is necessary to abate the hazard. Such an approach would be consistent with the Agency's previous description of non-emergency situations of environmental contamination as situations which do not require immediate action, but nevertheless reasonably support the conclusion of 'substantial risk.'" (Ref. 4). EPA believes that revised Part V.(b)(1), the reinstated Part V.(c), and the reporting procedures adequately make the distinction described by CMA in that a "substantial risk" in this context is an "emergency incident of environmental contamination" that "seriously threatens" humans or the environment.

IV. Claims of Confidentiality for Data Submitted under TSCA Section 8(e)

In general, health and safety information submitted to EPA—even as confidential—may be released to the

public, except as noted below. EPA considers information contained in a notice of substantial risk under TSCA section 8(e) to be health and safety information and, therefore, covered by the term "health and safety study," as defined in section 3(6) of TSCA. TSCA section 3(6) defines a "health and safety study" as "any study of any effect of a chemical substance or mixture on health or the environment or on both, including the underlying data and epidemiological studies, studies of occupational exposure to a chemical substance or mixture, toxicological, clinical, and ecological studies of a chemical substance or mixture, and any test performed pursuant to this Act."

Under TSCA section 14(b), health and safety information may be disclosed to the public (i.e., may not be protected as confidential). However, the section does not authorize public release of information concerning the manufacturing process of a chemical substance or mixture which is the subject of submitted health and safety information, including data "disclosing the portion of the mixture comprised by any of the chemical substances in the mixture."

In the legislative history of TSCA, the Conference Committee stated that "[i]t is intended that the term (health and safety studies) be interpreted broadly. Not only is information which arises as a result of a formal, disciplined study included, but other information relating to the effects of a chemical substance or mixture on health and the environment is also included. Any data that bears on the effects of a chemical substance on health or the environment would be included." (Ref. 5). EPA believes that TSCA section 8(e) information, such as information or underlying data from studies carried out to investigate the effects of a chemical (or a mixture of chemicals) on health or the environment, or reports concerning the effects of unintentional or accidental releases or exposures, is information that "bears on the effects of a chemical substance on health or the environment."

Therefore, incident information, exposure studies, and their underlying data should be considered covered under the term "health and safety study." To the extent that information contained in a section 8(e) substantial risk report falls within the meaning of the term "health and safety study" under TSCA, it will not be afforded TSCA "Confidential Business Information" (CBI) protection except as noted in the following paragraph.

EPA considers chemical identity to be part of, the underlying data to, a health and safety study. See, for example, 40 CFR 716.3 and 40 CFR 720.3(k). Consequently, the confidential identity of a chemical substance will not be protected by EPA unless otherwise provided for under section 14 of TSCA and the interpreting regulations in 40 CFR part 2.

EPA urges persons submitting data under TSCA section 8(e) to observe the limitations imposed on CBI claims by section 14 and the applicable regulations at 40 CFR part 2, subpart B, in order to save both Agency and submitter resources.

V. References

The following is a listing of the documents that are specifically cited in this guidance document, and which are available as part of the public docket described in Unit I.B.1.:

1. Monsanto Company. Letter from J. Ronald Condray. Comment #12. May 3, 1995.

2. United States Congress. (1976) Report of the Senate Committee on Commerce on S. 3149, No. 94–698: 8.

3. Chemical Manufacturers Association (CMA). Comments of the Chemical Manufacturers Association on TSCA Section 8(e) Notice of Clarification. October 28, 1993.

4. Chemical Manufacturers Association (CMA). Comments of the Chemical Manufacturers Association on TSCA Section 8(e) draft policy statement. Comment #6, p. 24. May 4, 1995.

5. United States Congress. (1976) House of Representatives, 94th Congress, 2d Session. H.R. Report 94–1679 (Conference Report and Debates): 58.

VI. Statutory and Executive Order Reviews

As discussed in Unit II.B., the guidance document articulates EPA's preferences for how and where TSCA section 8(e) notices should be submitted. The guidance document is not a regulation, and submitters of TSCA section 8(e) notices are not obligated to comply with the preferences. Since this document is not a regulation and does not impose any new binding requirements it is not subject to review by the Office of Management and Budget (OMB) under Executive Order 12866, entitled *Regulatory Planning and Review* (58 FR 51735, October 4, 1993), Executive Order 13045, entitled *Protection of Children from Environmental Health Risks and Safety Risks* (62 FR 19885, April 23, 1997), or Executive Order 13211, entitled *Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use* (66 FR 28355, May 22, 2001). For the same reason, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*) do not apply.

Pursuant to the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, an agency may not conduct or sponsor, and a person is not required to respond to, an information collection request as defined by the PRA, unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations, after appearing in the **Federal Register**, are listed in 40 CFR part 9 and 48 CFR chapter 15, and included on the related collection instrument or form, if applicable.

This document does not contain any new information collection requirements that would require additional OMB review and approval under the PRA. The information collection activities related to the submission of information pursuant to TSCA section 8(e) have been approved by OMB under OMB control number 2070–0046 (EPA ICR No. 0794). The annual respondent burden for this information collection activity is estimated to average 27 hours per initial section 8(e) submission and 5 hours per follow-up/supplemental section 8(e) submission, which includes the average time for processing, compiling and reviewing the requested data, generating the request, follow-up correspondence with EPA, storing, filing, and maintaining the data.

As defined by the PRA and 5 CFR 1320.3(b), "burden" means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

This document will have a negligible impact on States, local or Tribal governments because they do not generally engage in activities that would subject them to reporting requirements under TSCA section 8(e). Further this guidance document imposes no requirements on any entities, and instead is announcing Agency policies

and interpretations that generally will ease the reporting burdens under section 8(e). This action will not have substantial direct effects on State or tribal governments, on the relationship between the Federal government and States or Indian tribes, or on the distribution of power and responsibilities between the Federal government and States or Indian tribes. As a result, no action is required under Executive Order 13132, entitled *Federalism* (64 FR 43255, August 10, 1999), or under Executive Order 13175, entitled *Consultation and Coordination with Indian Tribal Governments* (65 FR 67249, November 6, 2000). Nor does it impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (Public Law 104–4).

This action requires no special considerations under Executive Order 12898, entitled *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (59 FR 7629, February 16, 1994); or Executive Order 12630, entitled *Governmental Actions and Interference with Constitutionally Protected Property Rights* (53 FR 8859, March 15, 1988).

This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note).

VII. Specific Revisions to the Policy Statement

For the reasons discussed in Unit III., EPA is making the following specific changes to the 1978 Policy Statement:

1. Part II. Persons Subject to the Requirement is amended by revising the note at the end of Part II.

2. Part IV. Requirement That a Person "Immediately Inform" the Administrator, Part VII. Information Which Need Not Be Reported, and Part IX. Reporting Requirements are revised.

3. Part V. What Constitutes Substantial Risk is amended by revising the heading of paragraph (b) and paragraph (b)(1) and adding the paragraph heading "Environmental effects." to the beginning of paragraphs (b)(2) through (b)(5).

VIII. [redacted] 8(e) Policy Statement and Guidance

As discussed previously, the following is a republication of the entire TSCA section 8(e) Policy Statement and Guidance, as amended:

[redacted]

The definitions set forth in TSCA section 3 apply to this policy statement. In addition, the following definitions are provided for purposes of this policy statement:

The term *manufacture or process for commercial purposes* means to manufacture or process: (1) For distribution in commerce, including for test marketing purposes, (2) for use as a catalyst or an intermediate, (3) for the exclusive use by the manufacturer or processor, or (4) for product research and development.

The term *person* includes any natural person, corporation, firm, company, joint-venture, partnership, sole proprietorship, association, or any other business entity, any State or political subdivision thereof, any municipality, any interstate body and any department, agency, or instrumentality of the Federal Government.

The term *substantial-risk information* means information which reasonably supports the conclusion that a chemical substance or mixture presents a substantial risk of injury to health or the environment.

II. [redacted]

Persons subject to section 8(e) requirements include both natural persons and business entities engaged in manufacturing, processing, or distributing in commerce a chemical substance or mixture. In the case of business entities, the president, chief executive officer, and any other officers responsible and having authority for the organization's execution of its section 8(e) obligations should ensure that the organization reports substantial risk information to EPA. The business organization is considered to have obtained any information which any officer or employee capable of appreciating the significance of that information has obtained. It is therefore incumbent upon business organizations to establish procedures for expeditiously processing pertinent information consistent with the schedule set forth in Part IV.

Those officers and employees of business organizations who are capable of appreciating the significance of pertinent information are also subject to these reporting requirements. An employing organization may relieve its individual officers and employees of any responsibility for reporting substantial-risk information directly to EPA by establishing, internally publicizing, and affirmatively implementing procedures for employee submission and corporate processing of pertinent information. These procedures, at a minimum, should: (1) Specify the information that officers and employees must submit; (2) indicate how such submissions are to be prepared and the company official to whom they are to be submitted; (3) note the Federal penalties for failing to report; and (4) provide a mechanism for promptly advising officers and employees in writing of the company's disposition of the report, including whether or not the report was submitted to EPA (and if not reported, informing employees of their right to report to EPA, as protected by TSCA section 23). An employee of any company that has established and publicized such procedures, who has internally submitted pertinent information in accordance with them, shall have discharged his section 8(e) obligation. [redacted] **notwithstanding, all officials responsible and having authority for the organization's execution of its section 8(e) obligations retain personal liability for ensuring that the appropriate substantial-risk information is reported to EPA.**

Business organizations that do not establish such procedures cannot relieve their individual officers and employees of the responsibility for ensuring that substantial-risk information they obtain is reported to EPA. While officers and employees of such organizations may also elect to submit substantial-risk information to their superiors, for corporate processing and reporting, rather than to EPA directly, they have not discharged their individual section 8(e) obligation until EPA has received the information.

Note: Irrespective of a business organization's decision to establish and publicize procedures described above, the business organization is responsible for becoming cognizant of any "substantial risk" information obtained by its officers, employees, and agents, and for ensuring that such information is properly reported to EPA.

III. When a Person Will Be Regarded as Having Obtained Information

A person obtains substantial-risk information at the time he first comes into possession of or knows of such information.

Note: This includes information of which a prudent person similarly situated could reasonably be expected to possess or have knowledge. An establishment obtains information at the time any officer or employee capable of appreciating the significance of such information obtains it.

[redacted] "immediately inform" the Administrator

With the exception of certain information on emergency incidents of environmental contamination (see Part V.(c)) and information submitted under Part VII. (c), (d) and (e), a person has "immediately informed" the Administrator if information is received by EPA not later than the 30th calendar day after the date the subject person obtained such information. Supplementary information concerning [redacted] after a [redacted] (within 30 calendar days of a person obtaining the information). This also applies to submitter responses to EPA requests for additional information related to submitted section 8(e) data. Section 8(e) reporting must be submitted to EPA and should be made as described under Part IX. For emergency incidents of environmental contamination, a person should report by telephone to the appropriate contact as directed in Part IX. as soon as the person has knowledge of the incident. The emergency incident report should contain as much of the information specified in Part IX. as is possible. A follow-up written report is not required.

Note: Preexisting information (i.e., of the kind described under Part V. (b)(1) and (c)) that predates June 3, 2003, is not subject to section 8(e) reporting unless its review is triggered by a person obtaining new information and that in combination with the preexisting information meets the criteria for section 8(e) reporting.

V. What Constitutes Substantial Risks

A "substantial risk of injury to health or the environment" is a risk of considerable concern because of (a) the seriousness of the effect (see subparts (a), (b), and (c) of this part for an illustrative list of effects of concern), and (b) the fact or probability of its occurrence. (Economic or social benefits of use, or costs of restricting use, are not to be considered in determining whether a risk is "substantial.") These two criteria are differentially weighted for different types of effects. The human health effects listed in subpart (a) of this part, for example, are so serious that relatively little weight is given to exposure: The mere fact the implicated chemical is in commerce constitutes sufficient evidence of exposure. In contrast, the remaining effects listed in subparts (b) and (c) of this part must involve, or be accompanied by the potential for, significant levels of exposure (because of general production levels, persistence, typical uses, common means of disposal, or other pertinent factors).

Note that information on the effects outlined below should not be reported: (i) If the respondent has actual knowledge that the Administrator is already informed of them, or (ii) information respecting these effects can be obtained either directly by observation of their occurrence, or inferred from designed studies as discussed in Part VI.

The Agency considers effects for which substantial-risk information should be reported to include the following.

(a) *Human health effects.* (1) Any instance of cancer, birth defects, mutagenicity, death, or serious or prolonged incapacitation, including the loss of or inability to use a normal bodily function with a consequent relatively serious impairment of normal activities, if one (or a few) chemical(s) is strongly implicated.

(2) Any pattern of effects or evidence which reasonably supports the conclusion that the chemical substance or mixture can produce cancer, mutation, birth defects or toxic effects resulting in death, or serious or prolonged incapacitation.

(b) *Non-emergency situations involving environmental contamination; environmental effects*—(1) *Non-emergency situations of chemical contamination involving humans and/or the environment.* Information that pertains to widespread and previously unsuspected distribution in environmental media of a chemical substance or mixture known to cause serious adverse effects, when coupled with information that widespread or significant exposure to humans or non-human organisms has occurred or that there is a substantial likelihood that such exposure will occur, is subject to reporting. The mere presence of a chemical in an environmental media, absent the additional information noted above, would not trigger reporting under section 8(e). Information concerning the detection of chemical substances contained within appropriate disposal facilities such as treatment, storage and disposal facilities permitted under RCRA should not be reported under this part.

Note: From time to time EPA establishes concentrations of various substances in different media that trigger a regulatory response or establish levels that are presumed to present no risk to human health or the environment. For example, EPA establishes Maximum Contaminant Levels (MCLs) in drinking water, Ambient Water Quality Criteria for receiving bodies of water, and Reference Doses (RfDs) or Concentrations (RfCs). For the purposes of section 8(e), information about contamination found at or below these kinds of benchmarks would not be reportable. Conversely, information about contamination found at or above benchmarks that trigger regulatory requirements, such as Resource Conservation and Recovery Act (RCRA) Toxicity Characteristic Limits, is to be considered for possible reporting, based on potential exposure to humans and/or non-human organisms and other relevant factors.

(2) *Environmental effects.* Measurements and indicators of pronounced bioaccumulation heretofore unknown to the Administrator (including bioaccumulation in fish beyond 5,000 times water concentration in a 30-day exposure or having an n-octanol/water partition coefficient greater than 25,000) should be reported when coupled with potential for widespread exposure and any non-trivial adverse effect.

(3) *Environmental effects.* Any non-trivial adverse effect, heretofore unknown to the Administrator, associated with a chemical known to have bioaccumulated to a pronounced degree or to be widespread in environmental media, should be reported.

(4) *Environmental effects.* Ecologically significant changes in species' interrelationships; that is, changes in population behavior, growth, survival, etc. that in turn affect other species' behavior, growth, or survival, should be reported.

Examples include: (i) Excessive stimulation of primary producers (algae, macrophytes) in aquatic ecosystems, e.g., resulting in nutrient enrichment, or eutrophication, of aquatic ecosystems.

(ii) Interference with critical biogeochemical cycles, such as the nitrogen cycle.

(5) *Environmental effects.* Facile transformation or degradation to a chemical having an unacceptable risk as defined above should be reported.

(c) *Emergency incidents of environmental contamination.* Any environmental contamination by a chemical substance or mixture to which any of the above adverse effects has been ascribed and which because of the pattern, extent, and amount of contamination (1) seriously threatens humans with cancer, birth defects, mutation, death or serious or prolonged incapacitation, or (2) seriously threatens non-human organisms with large-scale or ecologically significant population destruction, should be reported.

VI. Nature and Sources of Information Which "Reasonably Supports the Conclusion" of Substantial Risk

Information attributing any of the effects described in Part V. of this policy statement to a chemical substance or

mixture should be reported if it is one of the types listed below and if it is not exempt from the reporting requirement by reason of Part VII. of this policy statement. A person should not delay reporting until he obtains conclusive information that a substantial-risk exists, but should immediately report any evidence which "reasonably supports" that conclusion. Such evidence will generally not be conclusive as to the substantiality of the risk; it should, however, reliably ascribe the effect to the chemical.

Information from the following sources concerning the effects described in Part V. will often "reasonably support" a conclusion of substantial risk. Consideration of corroborative information before reporting can only occur where it is indicated below.

(1) *Designed controlled studies.* In assessing the quality of information, the respondent should consider whether it contains reliable evidence ascribing the effect to the chemical. Not only should final results from such studies be reported, but also preliminary results from incomplete studies where appropriate. Designed, controlled studies include:

(i) *In vivo* experiments and tests.

(ii) *In vitro* experiments and tests.

Consideration may be given to the existence of corroborative information, if necessary to reasonably support the conclusion that a chemical presents a substantial risk.

(iii) Epidemiological studies.

(iv) Environmental monitoring studies.

(2) *Reports concerning and studies of undesigned, uncontrolled circumstances.* It is anticipated here that reportable effects will generally occur in a pattern, where a significant common feature is exposure to the chemical. However, a single instance of cancer, birth defects, mutation, death, or serious incapacitation in a human would be reportable if one (or a few) chemicals) was strongly implicated. In addition, it is possible that effects less serious than those described in Part V.(a) may be preliminary manifestations of the more serious effects and, together with another triggering piece of information, constitute reportable information; an example would be a group of exposed workers experiencing dizziness together with preliminary experimental results demonstrating neurological dysfunctions. Reports and studies of undesigned circumstances include:

(i) Medical and health surveys.

(ii) Clinical studies.

(iii) Reports concerning and evidence of effects in consumers, workers, or the environment.

VII. Information Which Need Not Be Reported

"Substantial risk" information need not be reported under section 8(e) if it:

(a) Is obtained in its entirety from one of the following sources:

(1) An EPA study or report.

(2) An official publication or official report (draft or final) published or made available to the general public by another Federal agency and any information developed by another Federal Agency as a result of a toxicological testing/study program, or site evaluation for chemical contamination, in which EPA is collaborating in the design, review, or evaluation of testing/sampling plans or resultant data.

(3) Scientific publications, including bibliographic databases, available electronically or in hard copy (e.g., Science, Nature, New England Journal of Medicine, Medline, Toxline, NIOSH RTECS, International Uniform Chemical Information Database (IUCLID), etc.).

(4) Scientific databases (e.g., Agricola, Biological Abstracts, Chemical Abstracts, Dissertation Abstracts, Index Medicus, etc).

(5) A news publication (i.e., newspaper, news magazine, trade press) with circulation in the United States.

(6) A radio or television news report broadcast in the United States.

(7) A public scientific conference or meeting held within the United States, provided that the information is captured accurately by way of a meeting transcript, abstract, or other such record, and has been cited in a bibliographic/abstract computerized data base, publication, or report of the type cited in paragraphs (a) (1), (2), (3), or (4) of this part within 90 days of a subject person obtaining such information.

(8) A public scientific conference sponsored or co-sponsored by EPA or at a conference where the subject information is presented by an EPA employee or contractor acting on behalf of EPA.

(b) Corroborates (i.e., substantially duplicates or confirms) in terms of, for example, route of exposure, dose, species, strain, sex, time to onset of effect, nature and severity of effect, a well-recognized/well-established serious adverse effect for the chemical(s) under consideration, unless such information concerns effects observed in association with emergency incidents of environmental contamination as described in Part V.(c) and thus should be considered for reporting under section 8(e).

(c) Is information that will be reported to EPA within 90 calendar days of obtaining the information for non-emergency information under Part V.(b)(1), immediately (i.e., as soon as the subject person has knowledge of the incident) for emergency information under Part V.(c), or within 30 calendar days of obtaining the information for the other types of information specified under Part V., pursuant to a mandatory reporting requirement of any statutory authority that is administered by EPA (including, but not limited to, the Toxic Substances Control Act; the Federal Water Pollution Control Act; the Clean Air Act; the Federal Insecticide, Fungicide, and Rodenticide Act; the Safe Drinking Water Act; the Marine Protection, Research, and Sanctuaries Act; the Comprehensive Environmental Response, Compensation, and Liability Act; the Resource Conservation and Recovery Act, the Pollution Prevention Act; the Emergency Planning and Community Right-to-Know Act).

(d) Is information that will be reported to a State within 90 calendar days of obtaining the information for non-emergency information under Part V.(b)(1), immediately (i.e., as soon as the subject person has knowledge of the incident) for emergency information under Part V.(c), or within 30 calendar days of obtaining the information for the other types of information specified under Part V., pursuant to a mandatory reporting requirement under any Federal statute administered by EPA for which implementation has been delegated to that State (e.g., National Pollutant Discharge Elimination System (NPDES) permit requirements), or pursuant to a mandatory reporting provision of an EPA-authorized State program established under a Federal statute administered by EPA, e.g., state RCRA programs.

(e) Is information that will be reported to the Federal government within 90 calendar days of obtaining the information for non-emergency site-specific contamination information under Part V.(b)(1) or immediately (i.e., as soon as the subject person has knowledge of the incident) for emergency information under Part V.(c), pursuant to a mandatory reporting requirement under any Federal statute.

(f) Is information of the kind under Part V. (b)(1) and (c) submitted to the Federal government or a state that is developed in connection with an authorized (by the relevant Federal or state authority) site remediation program.

(g) Is information of the kind under Part V. (b)(1) and (c) concerning a site under the control of another person who is subject to the section 8(e) reporting authority.

(h) Is information of the kind under Part V.(b)(1) and (c) concerning a non-United States site provided the person who obtains the information does not have reason to believe that there is a substantial likelihood that the contamination will cause environmental contamination, of a nature that would be reportable under Part V. (b)(1) and (c), to occur in an area in the United States.

VIII. Information First Received By a Person Prior to the Effective Date of TSCA

Any substantial risk information possessed by a person prior to January 1,1977, of which he is aware after that date should be reported within 60 days of publication of this policy statement. The Agency considers that a person is aware of:

(a) Any information reviewed after January 1, 1971, including not only written reports, memoranda and other documents examined after January 1, 1971, but also information referred to in discussions and conferences in which the person participated after January 7, 1977;

(b) Any information the contents of which a person has been alerted to by date received after January 1, 1977, including any information concerning a chemical for which the person is presently assessing health and environmental effects;

(c) Any other information of which the person has actual knowledge.

IX. Reporting Requirements

Notices should be delivered to the Document Processing Center (7407M), (Attn: TSCA Section 8(e) Coordinator), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 1200 Pennsylvania Avenue, NW., Washington, DC 20460–0001

A notice should:

(a) Be sent by certified mail, or in any other way permitting verification of its receipt by the Agency.

(b) State that it is being submitted in accordance with section 8(e).

(c) Contain the job title, name, address, telephone number, and signature of the person reporting and the name and address of the manufacturing, processing, or distribution establishment with which the person is associated.

(d) Identify the chemical substance or mixture (including, if known, the Chemical Abstract Service (CAS) Registry Number).

(e) Summarize the adverse effect(s) or risk(s) being reported, describing the nature and the extent of the effect(s) or risk(s) involved.

(f) Contain the specific source of the information together with a summary and the source of any available supporting technical data.

For emergency incidents of environmental contamination (see Part V.(c)), a person should report the incident to the Administrator or the National Response Center by telephone as soon as he/she has knowledge of the incident. The report should contain as much of the information specified by paragraphs (c) through (f) of this part as possible. If any new substantial risk information concerning the incident and reportable under TSCA section 8(e) is obtained, supplementary reporting by the person is required. A twenty-four hour emergency telephone number is:

The National Response Center, (800) 424–8802 or (202) 267–2675 in the Washington, DC metropolitan area.

Region I (Maine, Rhode Island, Connecticut, Vermont, Massachusetts, New Hampshire), (617) 223–7265.

Region II (New York, New Jersey, Puerto Rico, Virgin Islands), (201) 548–8730.

Region III (Pennsylvania, West Virginia, Virginia, Maryland, Delaware, District of Columbia), (215) 814–3255.

Region IV (Kentucky, Tennessee, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Florida), (404) 562–8700.

Region V (Wisconsin, Illinois, Indiana, Michigan, Ohio, Minnesota), (312) 353–2318.

Region VI (New Mexico, Texas, Oklahoma, Arkansas, Louisiana), (214) 655–6428.

Region VII (Nebraska, Iowa, Missouri, Kansas), (913) 281–0991.

Region VIII (Colorado, Utah, Wyoming, Montana, North Dakota, South Dakota), (800) 227–8917.

Region IX (California, Nevada, Arizona, Hawaii, Guam), (415) 972–4400.

Region X (Washington, Oregon, Idaho, Alaska), (206) 553–1263.

X. Confidentiality Claims

(a) EPA may release to the public health and safety data claimed confidential, including information submitted in a notice of substantial risk under section 8 (e) of TSCA. EPA will disclose any information claimed confidential only to the extent, and by means of the procedures, set forth in 40 CFR part 2 (41 FR 36902, September 1, 1976)

(b) If no claim accompanies the notice at the time it is submitted to EPA, the notice will be placed in an open file to be available to the public without further notice to the submitter.

(c) To assert a claim of confidentiality for information contained in a notice, the submitter must submit two copies of the notice.

(1) The first copy should be complete and unedited, clearly reflecting what specific information is being claimed confidential. This should be done on each page by placing brackets around the specific information in question together with a label such as "confidential," "proprietary," or "trade secret."

(2) The second copy should be identical to the first copy, but with all bracketed information blanked out within the brackets.

(3) Information within the first confidential copy of the notice will be disclosed by EPA only to the extent, and by means of the procedures, set forth in 40 CPR part 2. The second copy will be placed in an open file to be available to the public

(d) Any person submitting a notice containing information for which they are asserting a confidentiality claim should send the notice in a double envelope.

(1) The outside envelope should bear the same address outlined in Part IX. of this policy statement.

(2) The inside envelope should be clearly marked "To be opened only by the OPPT Document Control Officer."

(e) The submitter should substantiate any CBI claims by answering substantiation questions according to the instructions located in the TSCA section 8(e) website: http://www.epa.gov/opptintr/tsca8e/doc/cbi.htm

XI. Failure to Report Information

Section 15(3) of TSCA makes it unlawful for any person to fail or refuse to submit information required under section 8(e). Section 16 provides that a violation of section 15 renders a person liable to the United States for a civil penalty and possible criminal prosecution. Pursuant to section 17, the Government may seek judicial relief to compel submittal of section 8(e) information and to otherwise restrain any violation of section 8(e).

List of Subjects

Environmental protection, Chemicals, Hazardous substances, Reporting and recordkeeping requirements.

Dated: May 15, 2003.

Stephen L. Johnson,

Assistant Administrator for Prevention, Pesticides, and Toxic Substances.

[FR Doc. 03–13888 Filed 6–2–03; 8:45 am]

BILLING CODE 6560–50–S

MR 286659

Draft Final Report

TITLE: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

SPONSOR:
American Petroleum Institute (API)
1220 L Street, NW
Washington, DC 20005

TESTING FACILITY:
Huntingdon Life Sciences (HLS)
Princeton Research Center
100 Mettlers Road
East Millstone, NJ 08875-2360

SITE OF POSTMORTEM EVALUATIONS AND ANALYSES:
RTI International (RTI)
Center for Life Sciences and Toxicology
Health Sciences Unit
Post Office Box 12194, 3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

STUDY INITIATION DATE:	October 27, 2004
EPA EXPERIMENTAL START DATE:	January 12, 2005
IN-LIFE PERFORMANCE DATES:	December 23, 2004 – February 3, 2005
EPA EXPERIMENTAL COMPLETION DATE:	February 3, 2005
DRAFT FINAL REPORT DATE:	_____
RTI IDENTIFICATION NUMBER:	09189.000

Author:

Rochelle W. Tyl, Ph.D., DABT Date
Study Director/Research Director
Center for Life Sciences and Toxicology
RTI International

Approved:

Alan H. Staple, M.Sc. Date
Vice President
Health Sciences Unit
RTI International

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ABSTRACT

A total of 23 plug-positive female CD-1 mice each were distributed on gestational day (gd) 0 into the 0, 2000, 10,000, and 20,000 mg/m^3 groups; a total of 38 plug-positive CD-1 mice were distributed on gd 0 into the 30,000 mg/m^3 group. Exposures were for 6 hours/day on gd 5 through 16 for the 0-20,000 mg/m^3 groups and for 6 hours/day on gd 5 through 10 for the 30,000 mg/m^3 group. The females were weighed on gd 0 and daily on gd 5 through 17; feed consumption and clinical observations were also recorded daily. Clinical observations were recorded individually before and after each exposure period and recorded at least once, using general categories (e.g., few, some, most, all, etc.) during each exposure period. At scheduled necropsy on gd 17, dams were euthanized, with body weight, gravid uterine weight, liver weight, paired adrenal gland weights, and paired kidney weights recorded. Ovarian corpora lutea were counted and uterine total implantations, resorptions, late fetal deaths, and live fetuses recorded for each pregnant dam. Each live fetus was euthanized by intraperitoneal injection of sodium phenobarbital, sexed, and examined externally (including examination for cleft palate). Each fetus was then dissected by a ventral longitudinal cut; the thoracic and abdominal viscera were removed and retained in buffered neutral 10% formalin, and the carcass was skinned after blanching and retained in 70% ethanol, for possible subsequent further examination.

There were no differences across groups in maternal body weights or weight changes before, during, or after the exposure period, except for a significant decrease in body weight change from gd 12 to 13 at 2000 and 20,000 mg/m^3. There were no differences in maternal gravid uterine weight or in absolute or relative paired adrenal gland weight across groups. Absolute maternal liver weight was significantly increased at 10,000 mg/m^3; relative maternal liver weight was significantly increased at 2000, 10,000, and 20,000 mg/m^3. Clinical observations that appeared treatment related included labored breathing in 1 female each at 20,000 and 30,000 mg/m^3 and lacrimation in 1 female at 20,000 mg/m^3 and in 3 females at 30,000 mg/m^3. Maternal feed consumption exhibited reductions early in the exposure period in all groups and increases in the postexposure period.

There were no differences across groups for the numbers of ovarian corpora lutea, uterine implantation sites, resorptions, late fetal deaths or live fetuses per litter, or percent pre- or postimplantation loss. There were also no statistically significant differences in the number (or

%) of nonlive (resorptions plus late fetal deaths) or adversely affected (nonlive plus malformed) implantations/litter.

For live litters, there were no differences across groups in the number of live fetuses/litter, % male fetuses/litter, number of male and female fetuses/litter, or in average fetal body weight per litter for all fetuses or by sexes separately. There were no differences across groups for incidences of external malformation or variations by fetuses or by litter.

External fetal malformations included encephalocele in 1 fetus (in 1 litter) at 2000 mg/m^3, cleft palate in 2 fetuses (in 2 litters) at 0 mg/m^3 and in 1 fetus (in 1 litter) each at 2000, 10,000, and 20,000 mg/m^3 and in 7 fetuses (in 4 litters) at 30,000 mg/m^3, and gastroschisis in 1 female (in 1 litter) at 30,000 mg/m^3; this female also had cleft palate. Fetal external variations included abnormal rugae in the palatal midline in 1 fetus (in 1 litter) each at 10,000 and 20,000 mg/m^3, and hematomas of the face, head, neck, and shoulder at 0-20,000 mg/m^3.

In conclusion, the current study: (1) did not confirm the presence of ectopia cordis (observed in the EMBSI study) in any fetus in any litter of any group, and therefore this fetal finding is not related to maternal exposure to the test material; (2) did not confirm the presence of gastroschisis in fetuses at 10,000 mg/m^3 (observed in the EMBSI study), or at 2000 or 20,000 mg/m^3 (not observed in the EMBSI study nor in the present study); (3) gastroschisis was observed in 1 female fetus in 1 litter at 30,000 mg/m^3; she also exhibited severely reduced body weight and cleft palate and was part of a litter with 2 other fetuses with cleft palate; and (4) gastroschisis was observed in 1 fetus (out of 407; 0.24%) in 1 litter (out of 33 litters; 3.03%) at 30,000 mg/m^3 with increased incidence of cleft palate (likely from increased maternal corticosterone synthesis and release in response to the stress of induced narcosis at this atmospheric concentration). Maternal treatment-related clinical signs of distress were observed at 20,000 and 30,000 mg/m^3. The results of this study indicate effects on fetuses at 30,000 mg/m^3, most likely due to maternal toxicity.

OBJECTIVES

The purpose of this study was to provide maternal and developmental toxicity data relative to a 6- or 12-day exposure regimen of inhaled gasoline methyl tertiary butyl ether (MTBE) vapor condensate during early or major organogenesis in gravid mice. A developmental toxicity evaluation of gasoline MTBE vapor condensate by inhalation to mice was one of a series of tests required in accordance with the Alternative Tier 2 provisions of fuels and fuels additives health effects testing regulations (40 C.F.R. § 79; Oge 1998). The study involved whole-body inhalation exposure of timed-pregnant CD-1 mice for at least 6 hours/day, on gestational day (gd) 5 through 17, to baseline gasoline vapor condensate with 21.5% MTBE at target concentrations of 0, 2000, 10,000, and 20,000 mg/m^3 (the last is 50% of the lower explosive limit; ExxonMobil Biological Sciences Institute [EMBSI], 2002). This study was conducted with the same exposure concentrations for gd 5 through 16, plus 30,000 mg/m^3 for 6 hours/day on gd 5 through 10 in order to confirm and extend the findings observed in the EMBSI study (2002).

MATERIALS AND METHODS

Test Material and Dose Formulations

The test material, gasoline MTBE vapor condensate (MRD-00-713; "API 211BG with MTBE Vapor Condensate") was a colorless liquid and identified by the supplier (Chevron Global Technology Services Company, Richmond, CA) as Lot/Batch Number API 00-02. Information on identity, strength, purity, and composition of gasoline MTBE vapor condensate was provided by the Sponsor and documented in the raw data and in this final report (Appendix IV, protocol attachment). Methods of synthesis, fabrication, or derivation were documented by the Sponsor and located at API. The test material was stable and stored under ambient conditions in an outside solvent shed except when in use in the inhalation laboratory. The test substance was handled as a flammable liquid. Detailed information on chemical handling is provided in the MSDS attached to the protocol (Appendix IV). The HLS Draft Inhalation Report is presented in Appendix I.

Animals and Husbandry

The proposed test animals were Caesarean-originated Virus Antibody Free (VAF) Crl:CD-1® (ICR) BR outbred albino mice supplied by Charles River Laboratories, Inc., Raleigh,

NC. The use of live animals was requested by the Sponsor and required by U.S. EPA OPPTS Testing Guidelines (U.S. EPA, 1998). Alternative test systems are not available for the assessment of chemical effects on prenatal mammalian development. The Charles River CD-1® mouse has been the subject of choice on developmental toxicology contracts at RTI since 1976. Large historical databases for reproductive performance and prevalence of spontaneous malformations in control mice are available from studies conducted at RTI (currently based on over 70 control litters).

The actual dates of all major phases of the study are presented in Table A.

Table A. Study Schedule

Event	Dates
Females arrive at HLS:	December 23, 2004
Quarantine (14 days):	December 23, 2004 – January 5, 2005
Animals paired:	January 6-11, 2005
Dates of gd 0:	January 7-17, 2005
TSCA experimental start date:	January 12, 2005
Exposure dates: gd 5 through 10	January 12 – January 22, 2005
gd 5 through 16	January 12 – February 2, 2005
Scheduled termination (gd 17)	January 24 – February 3, 2005
TSCA experimental termination date:	February 3, 2005
Submission of draft data on test atmospheres to Sponsor:	February 10, 2005 (within 1 week after the last exposure date, February 3, 2005)

One hundred seventy (170) nulliparous female mice were ordered for this study and arrived at HLS on December 23, 2004. One hundred (100) male mice, 9-11 weeks old upon arrival at HLS (on August 31, 2004), of the same strain and from the same supplier, were received for the previous range-finding study, and the remaining 99 males were used as a male breeding colony for this study. If more than the ordered number of females was received, any extra animals were used to replace any animals with clinical signs, injury, and/or reduced feed consumption. If none of the animals had indicators during quarantine, then the animal(s) with the lowest or highest body weight(s) were not used on study. The 99 males were used to generate timed-mated animals for this definitive developmental toxicity study which required the mating of 170 female mice (1:1, with the subsequent addition of naïve females to males who

inseminated their original females) to generate 130 plug-positive females. Females were 7-9 weeks old at arrival and 9-11 weeks of age and ~20-35 g in weight on gd 0. One hundred seventy (170) females were required to generate 130 plug-positive females in 11 consecutive days; 130 plug-positive females (23/group for 4 groups and 38/group for the fifth group) were required to supply the optimal number (based on EPA's guidance; e.g., OPPTS 870.3600; U.S. EPA, 1996; for inhalation developmental toxicity studies) of pregnant animals and litters to assess any maternal and/or embryo/fetal toxicity to the test substance and to confirm and extend the fetal findings from the previous EMBSI study.

During an approximately 14-day quarantine/acclimation period at the HLS testing facility, animals were checked for viability twice daily. Prior to study assignment, all animals were examined to ascertain suitability for study. The HLS veterinarian formally released these animals for use by signature and date. Males and females were individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female were housed together. During cohabitation, male and female mice were housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). Each cage was fitted to secure a glass feeder jar with a stainless steel lid. Clean feed jars and fresh feed were provided at least weekly. After the gd 14 exposure (for Groups 1-4) or on the afternoon of gd 14 (Group 5), a stainless steel, perforated insert was placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. Females not undergoing daily exposures after gd 10 (Group 5) were removed from their home cage and placed in another suspended cage without feed to match as closely as possible the conditions of Group 1-4 females for the 6-hour exposure period. They were then returned to their home cage at the same time as the exposed females for feed measurement overnight. Feed (PMI 5002 Certified Meal) was available *ad libitum*, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer were maintained on file at the HLS testing facility, and there were no known contaminants found in the feed. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) was available *ad libitum* via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses were conducted by Elizabethtown Water Company to assure that water met standards specified under the EPA Federal Safe

Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS testing facility. There were no known contaminants that interfered with the objectives of this study. At all times, animals were housed, handled, and used according to the National Research Council Guide (NRC, 1996).

A 12-hour light/dark cycle was provided via automatic timer. Temperature and relative humidity were monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18 to 26°C for temperature and 30 to 70% relative humidity was maintained to the maximum extent possible (NRC, 1996).

Each animal was assigned a temporary identification number (designated on each cage) upon receipt. During the second week of the quarantine/acclimation period, the 170 females received were tail tattooed with consecutive numbers 1 through 170. The 99 remaining males had been tail tattooed during the range-finding study with consecutive numbers 1 through 100 (except 87). After selection for use on the study, mating, indication of copulation, and assignment to 1 of the five groups, each female was ear tagged with a number assigned by the HLS testing facility. This number, plus the study number, comprised the unique animal number for each animal. Each cage was provided with a cage card that was color coded for exposure level identification and contained the study and animal numbers.

It was anticipated that the concentration employed would not result in irritation or corrosion to the respiratory tract of the test animals. Animals were not subjected to undue pain or distress. All procedures used in this study were designed to avoid discomfort, distress, and pain to the animals. The HLS IACUC Protocol Review Subcommittee and the RTI IACUC reviewed the protocol and found it to be in compliance with appropriate animal welfare regulations.

Immediately prior to pairing, each female was weighed and subjected to a clinical examination. For breeding, 1 male with 1 female pairing was employed since other pairing patterns (e.g., 1 male with 2 females) may have resulted in an unacceptable number of plug-positive, nonpregnant females and/or sire effects. Individual females were placed in polycarbonate "shoebox" cages with stainless steel lids with singly-housed males. On the following morning and each morning thereafter, the females were examined for the presence of a vaginal copulation plug (Hafez, 1970). The day on which copulation plugs were found was designated as gd 0. Plug-positive females (dams) were individually housed until scheduled

sacrifice on gd 17. Plug-negative females were retained in the same male's cage and checked for plugs on successive mornings until insemination occurred or the treatment groups were filled, whichever came first. HLS staff evaluated females for vaginal copulation plugs until all groups were filled and then completed the exposure schedule. When all treatment groups were filled, the remaining females were sacrificed by asphyxiation with CO_2 (and examined for pregnancy status; 35 of the 40 were in fact pregnant). The males were also euthanized by HLS staff. The fate of all animals was fully documented.

Study Design

This study was conducted with 4 treatment groups and 1 vehicle control group. Groups 1-4 were each comprised of 23 plug-positive female mice and Group 5 comprised of 38 plug-positive female mice (Table B).

Table B. Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	23	12	5 through 16	0
2	23	12	5 through 16	2000
3	23	12	5 through 16	10,000
4	23	12	5 through 16	20,000
5	38	6	5 through 10	30,000

The exposure period for Group 5 at 30,000 mg/m^3 of gd 5 through 10 was selected to reduce the number of days of generation of test atmosphere at a concentration that is 75% of the lower explosive limit. In addition, the fetal malformations of interest are formed early in the embryonic period of gestation; gd 7-9 in the mouse (e.g., Rugh, 1968), so extending the exposure period to gd 17 was unnecessary.

The test substance was administered as a vapor in the breathing air of the animals. The test atmosphere was generated by an appropriate procedure determined during prestudy trials. The trials were performed (at least two 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform conditions throughout the exposure chambers. The whole-body exposure chambers each had a volume of approximately 1000 liters. The chambers were operated at a minimum flow rate of

200 liters per minute. The final airflow was set to provide at least 1 air change in 5 minutes (12 air changes/hour) and a T_{99} equilibrium time of at most 23 minutes. This chamber size and airflow rate was considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of each daily 6-hour exposure, all animals remained in the chamber for a minimum of the T_{99} equilibrium time. During this time, the chamber was operated at approximately the same flow rate using clean air only.

A nominal exposure concentration was calculated. The flow of air through the chamber was monitored using appropriate calibrated equipment. The test substance consumed during the exposure was divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each 6-hour exposure, measurements of airborne concentrations were performed in the animals' breathing zone at least 4 times using an appropriate sampling procedure and IR analytical procedure. Specified airborne test material concentrations were within +/- 10% of the target concentrations. One sample per chamber during the trials period and the treatment period was analyzed by gas chromatography to characterize at least 10 major components (comprising at least 80% by weight of the test substance) to show test substance stability and comparison between the neat liquid test substance and the vaporized test atmospheres. During the treatment period, particle size determinations were performed once per chamber using a TSI Aerodynamic Particle Sizer to confirm the absence of particulate test substance condensate in the exposure atmosphere.

Chamber temperature, humidity, airflow rate, and static pressure were monitored continuously and recorded every 30 minutes during exposure. Chamber temperature and relative humidity were maintained, to the maximum extent possible, between 20 to 24°C and 40 to 60%, respectively. Chamber oxygen levels (maintained at least 19%) were measured pretest and at the beginning, middle, and end of the study. Air samples were taken in the vapor generation area pretest and at the beginning, middle, and end of the study. Light (maintained approximately 30 foot-candles at 1.0 meter above the floor) and noise levels (maintained below 85 decibels) in the exposure room were measured pretest and at the beginning, middle, and end of the study. The minimum frequency of chamber activity during the treatment period is summarized below:

Activity	Frequency/Chamber
Measured test substance concentration	4X/day
Measured test substance characterization	1X
Particle size	1X
Temperature	13X/day
Relative humidity	13X/day
Airflow rate	13X/day
Static pressure	13X/day
Nominal test substance concentration (excluding the air control chamber)	1X/day
Rotation pattern of exposure cages	1X/day
Loading/unloading verification	1X/day

Plug-positive female mice (dams) were assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights and equal distribution of females mated to the same male among dose groups using data from gd 0. Females were exposed to gasoline MTBE vapor condensate or air 6 hours per day from gd 5 through 16 for Groups 1-4 and for gd 5 through 10 for Group 5. For each daily exposure, females were transferred to inhalation cages, and the cages were moved into the appropriate chambers for exposure. Following each daily exposure, females were transferred back to home caging for feed consumption measurements overnight.

Clinical observations of all animals were made once daily on gd 0 through 4 (prior to exposure period), on gd 11 through 17 or gd 17 (after exposure period), and twice daily (prior to and immediately after each daily exposure) throughout the exposure period (gd 5 through 10 or gd 5 through 16). In addition, during each daily exposure period, animals were observed at least once during each exposure. This was routinely performed near the middle of each exposure.

Dams were weighed in the mornings (prior to exposures for those days that exposures occurred) on gd 0 and 5 through 17. Maternal weight gains were calculated for gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15,15-16, 16-17, 5 through 10 or 5 through 16 (exposure period), 10 through 17 (postexposure period), and 0 through 17 (gestational period).

Maternal feed consumption was evaluated in the mornings from gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, 16-17, 5 through 10

or 5 through 16 (exposure period), 10 through 17 (postexposure period), and 0 through 17 (gestation period).

No maternal animals died during the course of the study. One female (No. 3814) at 10,000 mg/m^3 was removed from study due to a pre-existing condition. On gd 17, approximately 1 to 1½ days before expected parturition, all surviving maternal animals were killed by CO_2 asphyxiation by RTI staff. The thoracic and abdominal cavities and organs were examined, and pregnancy status was confirmed by uterine examination. Uteri that presented no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites that may have undergone very early resorption (Salewski, 1964). At sacrifice, the body, liver, uterus, paired adrenal glands, and paired kidneys of each plug-positive female were weighed. Ovarian corpora lutea were counted and uterine contents (i.e., number of implantation sites, early and late resorptions, dead fetuses, live fetuses) recorded.

Live and dead fetuses were removed from the uterus, counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each fetus was killed by intraperitoneal injection of sodium pentobarbital. Live and dead fetuses were dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled scintillation vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcass was blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses were destroyed by incineration.

Statistics

The unit of comparison was the pregnant female or litter. Quantitative continuous data (e.g., maternal body weights, feed consumption, fetal body weights, etc.) were compared among the 4 treatment groups and 1 vehicle control group using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967), which do not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's Test (Levene, 1960), which is more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test

all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences (via Wald Chi-Square Tests), followed by individual *t*-tests for exposed vs. control group comparisons when the overall treatment effect was significant. The presence of linear trends was analyzed by robust regression methods for nonhomogenous data. Robust regression methods are available in the REGRESS procedure of SUDAAN® Release 8. (RTI, 2001).

If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® Release 8 was used to evaluate the overall effect of treatment and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For the litter-derived percentage data, the ANOVA was weighted according to litter size. The presence of linear trends was analyzed by GLM procedures for homogenous data (SAS Institute Inc., 1999a,b,c,d,e; 2000; 2001). A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS® Release 8 (SAS Institute Inc., 1999a,b,c,d,e; 2000; 2001).

Nominal scale measures were analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group.

A test for statistical outliers (SAS Institute, Inc., 1999b) was performed on female body weights, feed consumption (in g/day), and selected organ weights. If examination of pertinent study data did not provide a plausible, biologically sound reason for inclusion of the data flagged

as "outlier," then the data were excluded from summarization and analysis and designated as outliers.

Storage of Records

All data documenting experimental details and study procedures and observations were recorded and maintained as raw data. At the completion of the study, all reports, raw data, preserved specimens, and retained samples will be maintained in RTI's secure archives for a period of 1 year after submission of the signed final report. The Sponsor will be contacted in order to determine the final disposition of these materials.

Personnel

This study was conducted at HLS (Mr. G.M. Hoffman, Principal Investigator; Animal Research Facility Veterinarian, Dr. Teresa S. Kusznir; Animal Research Facility Director, Mr. I. Vanterpool; Necropsy Laboratory Supervisor, Ms. G.E. Baxter; Inhalation Laboratory Supervisor, Mr. S. Cracknell; Analytical Chemistry, Ms. K. Saladdin; Quality Assurance, Ms. N.S. Iacono, under contract to the API (Mr. T.M. Gray, Sponsor's Representative). Dr. R.W. Tyl of RTI served as Study Director. RTI Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W.P. Ross, Mr. C.G. Leach, Ms. L.L. Macdonald, Ms. N.M. Kuney, and Ms. A.J. Parham. RTI Quality Assurance personnel were Ms. D.A. Drissel (Manager), Ms. C.A. Ingalls, Ms. M.M. Oh, and Ms. S.C. Wade.

The final report was prepared by Dr. R.W. Tyl and Ms. M.C. Marr, with assistance from Ms. C.B. Myers for statistical analyses and generation of tables, and by Mr. T.W. Wiley for data entry. Ms. M.C. Marr was responsible for all transfer of custody procedures for transfer of records and tissues from HLS to RTI, and for archiving the study records.

Compliance with Good Laboratory Practice Regulations and Quality Assurance Oversight

RTI International (RTI; Research Triangle Park, NC) was responsible for study design, protocol generation, necropsy of the maternal and fetal animals on gd 17, generation of summary and individual data tables, and study draft and final report generation (with RTI QA oversight).

RTI's Quality Assurance Unit performed a prestudy on-site inspection, reviewed the protocol and amendments, and monitored all phases of the study in which RTI personnel participated. Huntingdon Life Sciences (HLS; East Millstone, NJ) was responsible for receipt of the test substance, prestudy and study generation and analyses of the test vapors, receipt, quarantine, and housing of the test females and breeder males, mating and assignment of the study animals, in-life observations, loading and unloading study females into and out of chambers, and submission of interim and final inhalation reports. The Quality Assurance Unit of HLS reviewed the protocol and monitored the facilities, equipment, personnel, methods, practices, records, raw data, draft and final inhalation reports, and controls used in this study to assure that they were in conformance with company standard operating procedures and the referenced Good Laboratory Practice (GLP) regulations.

This study was conducted in accordance with the U.S. EPA's GLP standards for the 211(b) program (40 C.F.R. 79.60) and performed according to the protocol and HLS' and RTI's SOPs. This study complied with all appropriate parts of the USDA Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, *Federal Register*, Vol. 54, No. 168, August 31, 1989, pp. 36112-36163, effective October 30, 1989, and 9 CFR Part 3 Animal Welfare Standards; and the Final Rule, *Federal Register*, Vol. 55, No. 32, February 15, 1991, pp. 6426-6505, effective March 18, 1991, and U.S. EPA TSCA GLPs (U.S. EPA, 1989).

RESULTS

Atmosphere Generation and Analysis

The test atmospheres were generated to within 97.5 to 104% of the target (grand mean of daily means/chamber). There was no test material detected in the control chamber, with an estimated limit of quantification (LOQ) of 433 mg/m^3. The relative content of MTBE was 21-23% as provided by the supplier. The analytical profile of gasoline MTBE vapor condensate at HLS indicated 26-27% MTBE, confounded by coelution with 2,3-dimethylbutane using gas chromatography with a Flame Ionization Detector (FID) and a previously used Supelco Petrocol™ column on September 24, 2004, and ~23-25% MTBE (confounded by coelution with 3-methylpentane) using a new column on December 9, 2004. Net MTBE concentrations were 21.89-22.09% (Table 1 and Appendix I).

Maternal Toxicity

A total of 23 plug-positive dams were assigned on sd 0 by stratified randomization (by body weight) to each of 4 groups (0 [Group 1], 2000 [Group 2], 10,000 [Group 3], and 20,000 [Group 4] mg/m^3), and 38 plug-positive dams were similarly assigned to Group 5 (30,000 mg/m^3). One female (No. 3814) at 10,000 mg/m^3 was removed from study due to a pre-existing condition (right side undescended testis, seminal vesicle and prostate, left side ovary, oviduct, uterus, cervix and vagina). No females died or were euthanized moribund. The numbers of confirmed nonpregnant females (at scheduled sacrifice) were 0, 1, 3, 1, and 2 and fully resorbed litters were 0, 1, 0, 2, and 3 at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively. The number (and %) pregnant were 23 (100.0), 22 (95.7), 19 (86.4), 22 (95.7), and 36 (94.7) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively (Table 2).

There were no effects of exposure across all groups on maternal body weights for gd 0, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 (in-life), and 17 (at sacrifice). Maternal body weight changes were similarly unaffected across all groups for all intervals: gd 0-5 (pre-exposure period for all groups), gd 5-10 (exposure period for group 5), gd 5-16 (exposure period for Groups 1-4), gd 10-17 (postexposure period for Group 5), gd 16-17 (postexposure period for Groups 1-4), and gd 0-17 (gestation period). Maternal gestational weight change (gestational body weight gain minus gravid uterine weight) was also unaffected across groups (Table 2).

At scheduled necropsy on gd 17, maternal absolute gravid uterine weight, paired adrenal gland weight, and paired kidney weight were unaffected across all groups. Maternal absolute liver weight was equivalent across 0, 2000, 20,000, and 30,000 mg/m^3 and was significantly increased at 10,000 mg/m^3. Maternal paired adrenal gland and paired kidney weights (relative to terminal body weights) were equivalent across all groups. Relative maternal liver weight was significantly increased in a concentration-related manner at 2000, 10,000, and 20,000 mg/m^3 (all groups with exposures ending on gd 16); relative liver weight was unaffected at 30,000 mg/m^3, with exposures ending on gd 10 (Table 2).

Maternal clinical observations for gd 0-4 (pre-exposed) prior to and after each daily exposure period and postexposure (gd 16-17 for Groups 1-4 or gd 11-17 for Group 5) are presented in Table 3. Moderate alopecia on extremities/snout was observed starting on gd 5 in 1 female at 20,000 mg/m^3. Enophthalmos (eyeball sunk into orbital cavity), left, was observed in

1 female at 30,000 mg/m^3. Labored breathing was observed on gd 9 postexposure for 1 female at 20,000 mg/m^3 and on gd 10 postexposure for 1 female at 30,000 mg/m^3. Unilateral moderate lacrimation was observed in 1 female at 30,000 mg/m^3 beginning postexposure on gd 5, and 2 females (1 each at 20,000 and at 30,000 mg/m^3) beginning postexposure on gd 6. Bilateral moderate lacrimation was observed in 1 female at 30,000 mg/m^3, beginning postexposure on gd 6. Also, red exudates were observed from the anogenital area in 1 female each at 10,000 and 20,000 mg/m^3, beginning on gd 11 postexposure at 20,000 mg/m^3 and on gd 12 prior to exposure at 10,000 mg/m^3 (Table 3).

Maternal feed consumption (in g/day) was significantly reduced at 20,000 mg/m^3 for gd 0-5 (pre-exposure period), significantly increased at 10,000 mg/m^3 for gd 5-6, significantly increased at 2000 and 10,000 mg/m^3 for gd 6-7, significantly reduced at 20,000 mg/m^3 for gd 7-8, and significantly reduced at 20,000 and 30,000 mg/m^3 for gd 8-9. Feed consumption (in g/day) was significantly reduced at 30,000 mg/m^3 for gd 10-11, significantly increased at 10,000 mg/m^3 for gd 12-13, and significantly increased at 30,000 mg/m^3 for gd 13-14. Feed consumption in g/day was equivalent across all groups for gd 9-10, 11-12, 14-15, 15-16, 16-17 (postexposure period, Groups 1-4), gd 5-10 (exposure period only for Group 5, 30,000 mg/m^3), gd 5-16 (exposure period for Groups 1-4), gd 10-17 (postexposure period for Group 5), and gd 0-17 (gestational period) (Table 4).

Maternal feed consumption (in g/kg body weight/day) was significantly reduced at 20,000 mg/m^3 for gd 0-5 (pre-exposure period), significantly increased at 10,000 mg/m^3 for gd 5-6, significantly increased at 2000 and 10,000 mg/m^3 for gd 6-7, significantly reduced at 20,000 mg/m^3 for gd 7-8, 8-9, 9-10, and 11-12, significantly reduced at 30,000 mg/m^3 for gd 8-9, 10-11, and 5-10 (exposure period for Group 5), and significantly increased at 30,000 mg/m^3 for gd 13-14. There were no differences across groups for feed consumption (in g/kg/day) for gd 12-13, 14-15, 15-16, 16-17 (postexposure period for Groups 1-4), gd 5-16 (exposure period, Groups 1-4), gd 10-17 (postexposure period for Group 5), and gd 0-17 (gestation period) (Table 4).

Uterine and Embryofetal Findings

Maternal ovarian corpora lutea and uterine contents are presented in Table 5. For all pregnant females, there were no effects across groups for any reproductive parameter, including no effects on the numbers of ovarian corpora lutea/dam, uterine implantation sites/litter, percent preimplantation loss/litter, number of (or %) resorptions/litter, number (or %) of litters with resorptions, number (or %) of late fetal deaths/litter, number (or %) of litters with late fetal deaths, number (or %) of nonlive (late fetal deaths plus resorptions) implants/litter, number (and %) of litters with nonlive implants, number (and %) of litters with 100% nonlive implants (fully resorbed), number (or %) of adversely affected (nonlive plus malformed) implants/litter, and number (or %) of litters with adversely affected implants (Table 5).

For live litters, there were no effects across groups for the number of live fetuses/litter, percent male fetuses/litter, number of male or female fetuses/litter, and for average fetal body weight/litter for all fetuses or separately by sex (Table 5).

Summary and statistical analysis of fetal external malformations and variations are presented in Table 6. Presentation of fetal external malformations and variations by defect type is in Table 7. The number of fetuses (litters) examined were 276 (23), 236 (21), 225 (19), 252 (20), and 407 (33) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively. There were no differences across groups for any of the parameters evaluated. They included the number and percentage of fetuses with external malformations per litter (total and separately by sex) and the number and percentage of fetuses and litters with external malformations. Also, there were no differences across groups for the same parameters as above for fetal external variations. There were fetal external malformations and variations observed in all 5 groups (Table 6).

The fetal external malformations included encephalocele in 1 fetus in 1 litter at 2000 mg/m^3, cleft palate in 2 fetuses (2 litters), 1(1), 1(1), 1(1), and 7(4) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3 respectively, and gastroschisis in 1 fetus (in 1 litter) at 30,000 mg/m^3.

The fetal external variations included abnormal rugae in the midline of the palate in 1 fetus (in 1 litter) each at 10,000 and 20,000 mg/m^3 and hematomas at various locations (face, head, neck, and shoulder) at 0, 2000, 10,000, and 20,000 mg/m^3 (Table 7).

DISCUSSION

This study was designed and performed:

1. To confirm or refute the fetal malformation finding of ectopia cordis observed in 1 fetus at 2000 mg/m^3 and in 2 fetuses (in the same litter) at 10,000 mg/m^3 in the previous developmental toxicity study on this test material in CD-1® mice at EMBSI;
2. To confirm or refute the fetal malformation finding of gastroschisis observed in 1 fetus at 10,000 mg/m^3 (but not at 2000 or 20,000 mg/m^3) in the previous developmental toxicity study at EMBSI on this test material in CD-1® mice;
3. To extend the test atmospheric concentration range from 0, 2000, 10,000, and 20,000 mg/m^3 on gd 5 through 16 employed by EMBSI, to 0, 2000, 10,000, 20,000, and 30,000 mg/m^3 (the last concentration at 75% of the lower explosive limit), with daily exposures on gd 5 through 16 for the 0-20,000 mg/m^3 groups and on gd 5 through 10 for the 30,000 mg/m^3 group (the last to encompass the time of embryonic ventral wall closure, the failure of which is likely responsible for both the ectopia cordis and gastroschisis). There were 23 plug-positive fetuses/group at 0-20,000 mg/m^3 and 38 plug-positive fetuses at 30,000 mg/m^3 to improve the possibility of detection of these rare fetal malformations.

There was no effect on maternal body weights or weight gains and no consistent treatment- or concentration-related effects on maternal feed consumption. The treatment-related increases in absolute and relative maternal liver weights are most likely due to the induction of hepatic metabolizing enzymes, with the concomitant increase in liver weight (Conney, 1967). This is not considered maternal toxicity, per se, but an adaptive metabolic response to exposure to a xenobiotic. Maternal adrenal gland weights were not changed across groups, although the current thinking is that there is increased maternal production of corticosterone (causing fetal cleft palate) in response to the stress of moving the animals in and out of chambers in all groups and in the high "dose" group also from the stress of the narcotic effect of MTBE at this concentration (see Bevan et al., 1997a,b). Interestingly, lethargy was observed in the females at 30,000 mg/m^3 in the range-finding study but was not documented in this study during the daily exposures; it is likely the admittedly subjective effect was present in this study since it was

present in the range-finding study at the same exposure concentration and duration. Clinical observations of the dams also indicated treatment-related findings, e.g., labored breathing only at 20,000 and 30,000 mg/m^3, 1 female in each group, and lacrimation in 1 female at 20,000 mg/m^3 and in 3 females at 30,000 mg/m^3.

The CD-1® (Swiss) mice used by EMBSI were from the Charles River, Portgage, MI, facility; the CD-1® (Swiss) mice used in the current study were from the Charles River, Raleigh, NC, facility, because RTI International has a historical control database for developmental toxicity studies on this mouse strain from this source, and to preclude the possibility that the fetal findings from the EMBSI study were due to a spontaneous rate of these two fetal malformations in the Portage colony (due to founder effects, genetic drift, etc.). Females like the pseudohermaphroditic adult female at 10,000 mg/m^3 (and removed from study) have been observed at very low incidence in other studies with this mouse strain at RTI International from the Charles River, Raleigh, NC, facility.

1. The present study did <u>not</u> confirm the presence of ectopia cordis in any mouse fetus at any exposure concentration out of a total 122 litters and 1396 fetuses. In the absence of a clear dose-response pattern to this finding in the EMBSI study and the total absence of this finding in the present study, it is the Study Director's opinion that it is appropriate (and prudent) to conclude that this fetal finding is <u>not</u> related to maternal exposure to the test material.
2. The present study did not confirm the presence of gastroschisis in fetuses at 10,000 mg/m^3; it was not found at 2000 or 20,000 mg/m^3 at EMBSI, and it was also not found at 2000, 10,000, or 20,000 mg/m^3 in the present study. One fetus (out of 407) at 30,000 mg/m^3 in the present study did exhibit gastroschisis. This fetus (No. 6 female) was from Female No. 5810; her litter included 15 implants and 14 live fetuses. In her litter, No. 5 female and No. 12 male also exhibited cleft palates and No. 6 female had cleft palate as well as gastroschisis. In this group, at 30,000 mg/m^3, there were 7 fetuses in 4 litters with cleft palate (greater incidence relative to other four groups), with 3 of them in this index litter. The body weight of the single fetus with gastroschisis and cleft palate was much lower (0.6057 g) than the body weights of the remaining fetuses in the litter: females 0.8034-0.9768 g; males 0.8406-0.8893 g (Table A-4 in Appendix II). Her body

weight was also much lower than the mean female fetal body weight/litter for this group (1.0141±0.0239 [S.E.M.] g;) (Table 5). This group also contained 3 fully resorbed litters (out of 36 pregnant). Two litters were fully resorbed (out of 22 pregnant) at 20,000 mg/m^3, with 1 fetus in 1 litter with cleft palate (and no incidence of gastroschisis). There were no fully resorbed litters at 0 or 10,000 mg/m^3 and 1 fully resorbed litter at 2000 mg/m^3 (Table 2), with cleft palate incidence of 2 fetuses in 2 litters at 0 mg/m^3 and 1 fetus in 1 litter each at 2000, 10,000, and 20,000 mg/m^3 (Table 7).

In the present study, gastroschisis was observed in only 1 fetus, only at 30,000 mg/m^3 and only in the presence of profound fetal toxicity for that fetus (very low body weight and cleft palate). Historical control data on the Charles River CD-1® (Swiss) mouse at RTI (Appendix V) indicates no gastroschisis in 71 litters (841 fetuses). No other historical control data on maternal and fetal findings in the Charles River CD-1® mouse could be found in the open literature. Neither gastroschisis nor ectopia cordis were observed in CD-1® mouse fetuses from mothers exposed to 0, 1000, 4000, or 8000 ppm MTBE (in the presence of maternal and embryofetal toxicity at 4000 and 8000 ppm MTBE). It appears obvious that exposure to MTBE by itself does not cause ectopia cordis or gastroschisis in mice. Maternal ataxia, hypoactivity, prostration, labored breathing, and lacrimation were observed at 4000 and 8000 ppm, and the resultant elevated circulating corticosteroids from stress were most likely responsible for the increased incidence of cleft palate. At 8000 ppm, reduced fetal body weights and concomitant reduced fetal skeletal ossification were observed at 4000 and 8000 ppm (Bevan et al., 1997a). Neither gastroschisis nor ectopia cordis were observed in CD® rat offspring in a 2-generation study of inhaled MTBE at 400, 3000, or 8000 ppm (Bevan et al., 1997b) or in rabbit fetuses from does exposed to 1000, 4000, or 8000 ppm MTBE (Bevan et al., 1997a).

Therefore, in the Study Director's opinion, maternal exposure to the test chemical at extremely high atmospheric concentrations, in the presence of fetal and demonstrable maternal toxicity, during the embryonic period of ventral body wall closure, results in a very low incidence of gastroschisis (1 out of 407 fetuses, 0.24%; 1 out of 33 litters with live fetuses, 3.03%) in genetically and systemically vulnerable mouse fetuses. With lower fetal and maternal toxicity at 20,000 mg/m^3, there was no incidence of gastroschisis.

The incidence of fetal cleft palate in the EMBSI study was only 1 fetus (in 1 litter), and only at 20,000 mg/m^3 (that study's highest concentration). In the present study, cleft palate was observed in all 5 groups, including the air control group (2 fetuses in 2 litters), at 2000-20,000 mg/m^3 (1 fetus in 1 litter in each group) and at 30,000 mg/m^3 (7 fetuses in 4 litters). Cleft palate in fetal mice is inducible by increased corticosterone levels in the dam (and presumably transported to the fetuses). Maternal increased corticosterone levels are due to increased maternal stress from inhalation exposures, per se (moving dams into and out of chambers, exposure to dynamic air flows, no feed or water during exposure periods, no solid flooring in exposure cages, etc.), and from test materials with anesthetic qualities. In fact, maternal inhalation of MTBE has been shown to produce cleft palates in fetuses from CD-1 mouse dams exhibiting lethargy and apparent unconsciousness (Bevan et al., 1997a). Maternal lethargy during exposures was also observed by HLS staff during the daily exposure periods at 30,000 mg/m^3 in the range-finding study at HLS (it was not noted by HLS staff during the daily exposure periods at any concentration in this definitive study). Therefore, the presence of fetal cleft palate in all groups (including the control group) was not unexpected, and the increased incidence at 30,000 mg/m^3 (from both inhalation procedures, per se, and the anesthetic qualities of the MTBE in the gasoline MTBE vapor condensate at this atmospheric concentration) was also anticipated. The increased cleft palate incidence at 30,000 mg/m^3 is most likely due to maternal effects and is consistent with the presence of gastroschisis in vulnerable fetuses developing in compromised dams.

In conclusion, this study has demonstrated the following:

1. No confirmation of fetal ectopia cordis at any test atmospheric concentration employed;
2. No confirmation of fetal gastroschisis at 0-20,000 mg/m^3;
3. One fetus (out of 407 fetuses, 0.24%) in one litter (out of 33 litters with live fetuses, 3.03%) exhibited gastroschisis at 30,000 mg/m^3; this fetus had very low body weight and also exhibited cleft palate. This female fetus was clearly compromised and this mouse strain may be susceptible (i.e., it did exhibit a very low incidence of gastroschisis in the EMBSI study at 2000 and 10,000 mg/m^3). The presence of gastrochisis is consistent with the increased incidence of cleft

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Table 1. Analysis of Test Atmospheres (page 1 of 1)

	Target Concentrations (mg/m^3)				
	0	2000	10,000	20,000	30,000
Mean analytical concentration ± SD (% of target)[a]	0.00 ± 0.00 (NA)	2074 ± 248 (104)	9899 ± 700 (99.0)	20,297 ± 1815 (102)	29,250 ± 1480 (97.5)
Particle Size Determination:[b]					
MMAD (μm)	2.179	5.699	9.319	3.845	1.144
GSD	1.676	2.117	2.071	1.955	2.910
TMC (mg/m^3)	2.56×10^{-2}	5.05×10^{-3}	3.41×10^{-3}	1.47×10^{-3}	1.54×10^{-2}
Mean temperature (°C ± SD)[c]	20.3 ± 0.9	20.8 ± 1.2	21.5 ± 0.9	20.7 ±0.9	20.7 ±0.8
Mean relative humidity (% ± SD)[c]	31.2 ± 4.9	32.0 ± 7.1	26.7 ± 4.2	28.4 ± 4.1	27.6 ± 4.7

[a] Mean of 4 assays/chamber/day (20 days for Group 1, 18 days for Group 2, 22 days for Group 3, 19 days for Group 4, and 12 days for Group 5) measured by infrared spectroscopy

[b] Measured 1 time/chamber

[c] Measured 13 times/chamber/day

SD = Standard deviation

MMAD = mass median aerodynamic diameter

GSD = geometric standard deviation

TMC = total mass concentration (measure of aerosol concentration)

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 1 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Subjects (No. Dams)					
No. on Study	23	23	23	23	38
No. Removed	0	0	1[a]	0	0
No. Dead or Euthanized	0	0	0	0	0
No. Nonpregnant	0	1	3	1	2
No. (%) Pregnant at Scheduled Sacrifice	23 (100.0)	22 (95.7)	19 (86.4)	22 (95.7)	36 (94.7)
No. (%) with 100% Resorptions	0 (0.0)	1 (4.5)	0 (0.0)	2 (9.1)	3 (8.3)
Maternal Body Weight (gd 0) (g)[b]					
	26.7 ± 0.4 N=23	27.2 ± 0.3 N=22	27.0 ± 0.3 N=19	27.3 ± 0.3 N=22	27.5 ± 0.2 N=36
Maternal Body Weight (gd 5) (g)[b]					
	27.6 ± 0.4 N=23	28.4 ± 0.4 N=22	27.8 ± 0.4 N=19	28.5 ± 0.3 N=22	28.3 ± 0.3 N=36
Maternal Body Weight (gd 6) (g)[b]					
	28.2 ± 0.4 N=23	29.1 ± 0.4 N=22	28.7 ± 0.4 N=19	29.0 ± 0.3 N=22	28.8 ± 0.3 N=36
Maternal Body Weight (gd 7) (g)[b]					
	28.7 ± 0.4 N=23	29.7 ± 0.4 N=22	29.1 ± 0.4 N=19	29.6 ± 0.3 N=22	29.3 ± 0.3 N=36
Maternal Body Weight (gd 8) (g)[b]					
	29.2 ± 0.4 N=23	30.2 ± 0.4 N=22	29.7 ± 0.4 N=19	30.0 ± 0.3 N=22	29.8 ± 0.3 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 2 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight (gd 9) (g)[b]	29.7 ± 0.4 N=23	30.6 ± 0.5 N=22	30.3 ± 0.5 N=19	30.6 ± 0.4 N=22	30.4 ± 0.4 N=36
Maternal Body Weight (gd 10) (g)[b]	30.9 ± 0.5 N=23	31.8 ± 0.6 N=22	31.6 ± 0.5 N=19	31.5 ± 0.4 N=22	31.3 ± 0.4 N=36
Maternal Body Weight (gd 11) (g)[b]	32.8 ± 0.5 N=23	33.9 ± 0.6 N=22	33.6 ± 0.6 N=19	33.2 ± 0.5 N=22	33.0 ± 0.5 N=36
Maternal Body Weight (gd 12) (g)[b]	34.9 ± 0.5 N=23	35.8 ± 0.8 N=22	35.5 ± 0.5 N=19	35.1 ± 0.6 N=22	35.3 ± 0.5 N=36
Maternal Body Weight (gd 13) (g)[b]	36.9 ± 0.5 N=23	37.2 ± 0.9 N=22	37.3 ± 0.6 N=19	36.7 ± 0.7 N=22	37.4 ± 0.6 N=36
Maternal Body Weight (gd 14) (g)[b]	39.2 ± 0.6 N=23	39.2 ± 1.0 N=22	39.4 ± 0.6 N=19	38.7 ± 0.9 N=22	39.7 ± 0.7 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 3 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight (gd 15) (g)[b]	42.0 ± 0.6 N=23	42.0 ± 1.1 N=22	42.2 ± 0.7 N=19	41.2 ± 1.0 N=22	42.4 ± 0.9 N=36
Maternal Body Weight (gd 16) (g)[b]	45.2 ± 0.7 N=23	45.1 ± 1.4 N=22	45.3 ± 0.7 N=19	44.0 ± 1.2 N=22	45.2 ± 1.1 N=36
Maternal Body Weight (gd 17) (g)[b]	48.4 ± 0.7 N=23	48.0 ± 1.5 N=22	48.3 ± 0.8 N=19	46.7 ± 1.4 N=22	48.3 ± 1.2 N=36
Maternal Body Weight (gd 17 at sacrifice) (g)[b]	47.14 ± 0.73 N=23	46.71 ± 1.50 N=22	47.52 ± 0.79 N=19	45.91 ± 1.43 N=22	47.44 ± 1.18 N=36
Maternal Body Weight Change (gd 0 to 5) (g)[b]	0.8 ± 0.2 N=23	1.2 ± 0.3 N=22	0.8 ± 0.3 N=19	1.2 ± 0.2 N=22	0.8 ± 0.2 N=36
Maternal Body Weight Change (gd 5 to 6) (g)[b]	0.6 ± 0.1 N=23	0.7 ± 0.1 N=22	0.9 ± 0.2 N=19	0.5 ± 0.1 N=22	0.5 ± 0.1 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 4 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 6 to 7) (g)[b]					
#	0.6 ± 0.1 N=23	0.6 ± 0.1 N=22	0.5 ± 0.1 N=19	0.6 ± 0.1 N=22	0.5 ± 0.1 N=36
Maternal Body Weight Change (gd 7 to 8) (g)[b]	0.5 ± 0.1 N=23	0.5 ± 0.1 N=22	0.6 ± 0.1 N=19	0.4 ± 0.1 N=22	0.5 ± 0.1 N=36
Maternal Body Weight Change (gd 8 to 9) (g)[b]	0.5 ± 0.1 N=23	0.4 ± 0.1 N=22	0.6 ± 0.1 N=19	0.5 ± 0.1 N=22	0.6 ± 0.1 N=36
Maternal Body Weight Change (gd 9 to 10) (g)[b]	1.2 ± 0.1 § N=23	1.2 ± 0.1 N=22	1.3 ± 0.2 N=19	0.9 ± 0.1 N=22	0.9 ± 0.1 N=36
Maternal Body Weight Change (gd 10 to 11) (g)[b]	1.9 ± 0.1 N=23	2.0 ± 0.2 N=22	2.0 ± 0.1 N=19	1.7 ± 0.2 N=22	1.7 ± 0.1 N=36
Maternal Body Weight Change (gd 11 to 12) (g)[b]	2.1 ± 0.1 § N=23	1.9 ± 0.2 N=22	1.8 ± 0.1 N=19	2.0 ± 0.2 N=22	2.3 ± 0.1 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 5 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 12 to 13) (g)[b]	2.1 ‡‡ ± 0.1 N=23	1.4 ** ± 0.2 N=22	1.9 ± 0.1 N=19	1.5 * ± 0.2 N=22	2.0 ± 0.1 N=36
Maternal Body Weight Change (gd 13 to 14) (g)[b]	2.3 ± 0.1 N=23	2.0 ± 0.2 N=22	2.1 ± 0.1 N=19	2.0 ± 0.2 N=22	2.3 ± 0.2 N=36
Maternal Body Weight Change (gd 14 to 15) (g)[b]	2.8 ± 0.1 N=23	2.8 ± 0.2 N=22	2.8 ± 0.1 N=19	2.5 ± 0.2 N=22	2.7 ± 0.3 N=36
Maternal Body Weight Change (gd 15 to 16) (g)[b]	3.2 ± 0.2 N=23	3.1 ± 0.2 N=22	3.0 ± 0.1 N=19	2.8 ± 0.3 N=22	2.9 ± 0.2 N=36
Maternal Body Weight Change (gd 16 to 17) (g)[b]	3.2 ± 0.2 N=23	2.9 ± 0.2 N=22	3.1 ± 0.1 N=19	2.7 ± 0.2 N=22	3.1 ± 0.1 N=36
Maternal Body Weight Change (gd 5 to 10) (g)[b,c]	3.3 ± 0.2 N=23				3.0 ± 0.2 N=36
Maternal Body Weight Change (gd 5 to 16) (g)[b,d]	17.6 ± 0.4 N=23	16.7 ± 1.2 N=22	17.5 ± 0.5 N=19	15.5 ± 1.2 N=22	

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights, and Relative Organ Weights (page 6 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 10 to 17) (g)[b,c]	17.5 ± 0.4 N=23				17.0 ± 0.9 N=36
Maternal Body Weight Change (gestation) (g)[b]	20.4 ± 0.6 N=23	19.6 ± 1.3 N=22	20.5 ± 0.7 N=19	18.6 ± 1.5 N=22	20.0 ± 1.1 N=36
Maternal Body Weight Change (corrected) (g)[b,e]	3.14 ± 0.25 N=23	3.98 ± 0.44 N=22	3.95 ± 0.51 N=19	2.88 ± 0.38 N=22	3.20 ± 0.32 N=36
Gravid Uterine Weight (g)[b]	17.2900 ± 0.4737 N=23	15.5703 ± 1.1217 N=22	16.5732 ± 0.3737 N=19	15.7215 ± 1.2039 N=22	16.7807 ± 0.9127 N=36
Maternal Liver Weight (g)[b]	2.4511 ‡ ±0.0498 N=23	2.5766 ±0.0889 N=22	2.7247 * ±0.0538 N=19	2.6308 ±0.0715 N=22	2.4253 ±0.0604 N=36
Maternal Paired Adrenal Gland Weight (g)[b]	0.0136 ±0.0006 N=22[f]	0.0144 ±0.0007 N=22	0.0132 ±0.0004 N=18[f]	0.0137 ±0.0005 N=22	0.0135 ±0.0003 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights and Relative Organ Weights (page 7 of 8)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Paired Kidney Weight (g)[b]	0.4277 ±0.0089 N=23	0.4454 ±0.0107 N=22	0.4394 ±0.0054 N=19	0.4376 ±0.0089 N=22	0.4311 ±0.0073 N=36
Relative Maternal Liver Weight (% sacrifice weight)[b] #	5.1961 ††† ± 0.0569 N=23	5.5269 ÞÞ ± 0.0865 N=22	5.7418 ÞÞÞ ± 0.0927 N=19	5.7610 ÞÞÞ ± 0.0754 N=22	5.1550 ± 0.0939 N=36
Relative Maternal Paired Adrenal Gland Weight (% sacrifice weight)[b]	0.0290 ± 0.0014 N=22[f]	0.0314 ± 0.0016 N=22	0.0279 ± 0.0009 N=18[f]	0.0306 ± 0.0017 N=22	0.0297 ± 0.0015 N=36
Relative Maternal Paired Kidney Weight (% sacrifice weight)[b]	0.9067 ± 0.0109 N=23	0.9783 ± 0.0451 N=22	0.9281 ± 0.0156 N=19	0.9817 ± 0.0496 N=22	0.9345 ± 0.0337 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Body Weight Changes, Organ Weights. and Relative Organ Weights (page 8 of 8)

[a]Female 3814 was removed from study due to a pre-existing condition. At necropsy she was found to have an undescended testis on the right and seminal vesicles and prostate to the right of the vagina and cervix.

[b]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd=gestational day.

[c]This endpoint was only calculated for the 0 and 30,000 mg/m^3 dose groups.

[d]This endpoint was only calculated for the 0, 2000, 10,000, and 20,000 mg/m^3 dose groups.

[e]Weight change during gestation (gestational day 17 sacrifice weight minus gestational day 0 weight) minus gravid uterine weight.

[f]Decrease in N is due to the paired adrenal weight for one animal being a statistical outlier and therefore it was excluded.

#Levene's Test for homogeneity of variances was significant ($p<0.05$); therefore, robust regression methods were used to test all treatment effects.

‡$p<0.05$; ANOVA Test.

‡‡$p<0.01$; ANOVA Test.

§$p<0.05$; Test for Linear Trend.

*$p<0.05$; Dunnett's Test.

**$p<0.01$; Dunnett's Test.

†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.

ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.

ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

Table 3. Summary of the Maternal Clinical Observations (page 1 of 3)

A. Clinical Observations Summarized by Group

Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Alopecia: extremities/snout, moderate				1	
Eye: enophthalmos[a], unilateral, left					1
Labored breathing				1	1
Lacrimation, bilateral, moderate					1
Lacrimation, unilateral, moderate				1	2
Red exudates from anogenital area			1	1	

B. Clinical Observations Summarized by Group, Day, and Time

Day[b]	Time[c]	Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
			Dosed gd 5-16				Dosed gd 5-10
			0	2000	10,000	20,000	30,000
2	Prior	Eye: enophthalmos, unilateral, left					1
3	Prior	Eye: enophthalmos, unilateral, left					1
4	Prior	Eye: enophthalmos, unilateral, left					1
5	Prior	Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Lacrimation, unilateral, moderate					1
6	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Lacrimation, bilateral, moderate					1
		Lacrimation, unilateral, moderate				1	1

Table 3. Summary of the Maternal Clinical Observations (page 2 of 3)

B. Clinical Observations Summarized by Group, Day, and Time

Day[b]	Time[c]	Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
			Dosed gd 5-16				Dosed gd 5-10
			0	2000	10,000	20,000	30,000
7	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
8	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
9	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Labored breathing				1	
10	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Labored breathing					1
11	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Red exudates from anogenital area				1	
12	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Red exudates from anogenital area			1		
	Post	Alopecia: extremities/snout, moderate				1	

Table 3. Summary of the Maternal Clinical Observations (page 3 of 3)

B. Clinical Observations Summarized by Group, Day, and Time

Day[b]	Time[c]	Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
			Dosed gd 5-16				Dosed gd 5-10
			0	2000	10,000	20,000	30,000
13	Prior	Eye: enophthalmos, unilateral, left					1
14	Prior	Eye: enophthalmos, unilateral, left					1
15	Prior	Eye: enophthalmos, unilateral, left					1
16	Prior	Eye: enophthalmos, unilateral, left					1
17	Prior	Eye: enophthalmos, unilateral, left					1

[a]A sinking of the eyeball into the orbital cavity.

[b]Gestational day.

[c]Time is prior to dosing (prior) or after dosing (post).

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 1 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Dams	23	22	19	22	36
Maternal Feed Consumption (gd 0 to 5) (g/day)[a] #	6.1 ††† ± 0.2 N=17[b]	6.5 ± 0.4 N=18[b,c]	6.2 ± 0.4 N=11[b]	5.3 ÞÞ ± 0.2 N=16[b,c]	6.8 ± 0.4 N=25[b,c]
Maternal Feed Consumption (gd 5 to 6) (g/day)[a] #	6.1 † ± 0.3 Ÿ N=20[b,d]	6.7 ± 0.3 N=21[b]	7.7 Þ ± 0.7 N=16[b]	6.2 ± 0.2 N=21[d]	5.9 ± 0.1 N=32[b,d]
Maternal Feed Consumption (gd 6 to 7) (g/day)[a] #	6.1 †† ± 0.2 N=18[b,d]	7.8 Þ ± 0.7 N=19[b,c,d]	7.7 ÞÞÞ ± 0.4 N=17[b]	6.2 ± 0.3 N=17[b,d]	6.3 ± 0.2 N=32[b,c]
Maternal Feed Consumption (gd 7 to 8) (g/day)[a] #	7.0 ††† ± 0.4 ŸŸŸ N=21[d]	7.4 ± 0.3 N=21[d]	8.2 ± 0.7 N=19	6.2 Þ ± 0.2 N=18[b,d]	6.4 ± 0.2 N=34[c,d]
Maternal Feed Consumption (gd 8 to 9) (g/day)[a] #	7.6 ††† ± 0.5 ŸŸŸ N=21[b]	6.8 ± 0.3 N=19[c,d]	7.5 ± 0.4 N=16[b,c]	6.1 ÞÞ ± 0.2 N=20[b,d]	6.2 ÞÞ ± 0.1 N=33[b,c]
Maternal Feed Consumption (gd 9 to 10) (g/day)[a]	6.7 ‡‡‡ ± 0.2 §§ N=23	7.0 ± 0.3 N=21[b]	7.3 ± 0.2 N=18[b]	6.1 ± 0.2 N=20[b,c]	6.3 ± 0.1 N=35[b]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 2 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Feed Consumption (gd 10 to 11) (g/day)[a]					
#	6.9 ††† ± 0.2 ŸŸŸ N=19[b]	7.6 ± 0.3 N=22	7.5 ± 0.2 N=18[b]	6.3 ± 0.2 N=20[d]	6.2 ÞÞ ± 0.1 N=33[c,d]
Maternal Feed Consumption (gd 11 to 12) (g/day)[a]					
	7.7 ‡ ± 0.4 N=23	7.1 ± 0.2 N=21[b]	7.7 ± 0.3 N=18[b]	6.7 ± 0.2 N=20[b,d]	7.8 ± 0.3 N=33[b,c,d]
Maternal Feed Consumption (gd 12 to 13) (g/day)[a]					
	7.1 ‡‡ ± 0.2 N=23	7.3 ± 0.3 N=21[c]	8.0 * ± 0.3 N=19	6.9 ± 0.2 N=22	7.8 ± 0.2 N=34[c,d]
Maternal Feed Consumption (gd 13 to 14) (g/day)[a]					
	7.3 ‡‡ ± 0.1 § N=23	7.5 ± 0.3 N=20[b,c]	7.9 ± 0.2 N=18[b]	7.1 ± 0.3 N=21[b]	8.0 * ± 0.2 N=36
Maternal Feed Consumption (gd 14 to 15) (g/day)[a]					
	7.4 ± 0.1 N=23	7.4 ± 0.3 N=21[b]	7.8 ± 0.2 N=19	7.1 ± 0.3 N=22	7.6 ± 0.2 N=36
Maternal Feed Consumption (gd 15 to 16) (g/day)[a]					
	7.5 ‡ ± 0.2 § N=23	7.8 ± 0.3 N=22	7.9 ± 0.3 N=19	6.9 ± 0.2 N=22	7.2 ± 0.2 N=35[c]
Maternal Feed Consumption (gd 16 to 17) (g/day)[a]					
	7.6 ± 0.2 N=23	7.4 ± 0.3 N=22	7.8 ± 0.2 N=19	7.3 ± 0.2 N=22	7.5 ± 0.2 N=35[c]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 3 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Feed Consumption (gd 5 to 10) (g/day)[a,e]					
#	6.9 ± 0.4 N=20[f]				6.2 ± 0.1 N=28[f]
Maternal Feed Consumption (gd 5 to 16) (g/day)[a,g]					
	6.9 ± 0.2 N=17[f]	7.0 ± 0.3 N=14[f]	7.7 ± 0.3 N=12[f]	6.8 ± 0.3 N=17[f]	
Maternal Feed Consumption (gd 10 to 17) (g/day)[a,e]					
	7.3 ± 0.2 N=19[f]				7.6 ± 0.2 N=31[f]
Maternal Feed Consumption (gd 0 to 17) (g/day)[a]					
	6.5 ‡ ± 0.1 N=14[f]	6.8 ± 0.3 N=13[f]	7.0 ± 0.3 N=8[f]	6.1 ± 0.2 N=14[f]	6.9 ± 0.2 N=20[f]
Relative Maternal Feed Consumption (gd 0 to 5) (g/kg/day)[a]					
#	224.8 ††† ± 10.3 N=17[b]	230.4 ± 11.3 N=18[b,c]	223.1 ± 13.5 N=11[b]	189.4 ÞÞ ± 5.5 N=16[b,c]	241.5 ± 14.3 N=25[b,c]
Relative Maternal Feed Consumption (gd 5 to 6) (g/kg/day)[a]					
#	216.1 † ± 8.3 Ÿ N=20[b,d]	233.1 ± 9.7 N=21[b]	270.7 Þ ± 24.6 N=16[b]	217.3 ± 8.9 N=21[d]	207.5 ± 4.6 N=32[b,d]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 4 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 6 to 7) (g/kg/day)[a] #	214.6 †† ± 7.6 Ÿ N=18[b,d]	266.0 Þ ± 22.5 N=19[b,c,d]	264.7 ÞÞ ± 14.5 N=17[b]	213.3 ± 8.4 N=17[b,d]	218.1 ± 7.2 N=32[b,c]
Relative Maternal Feed Consumption (gd 7 to 8) (g/kg/day)[a] #	242.4 ††† ± 14.8 ŸŸŸ N=21[d]	247.6 ± 10.4 N=21[d]	275.4 ± 21.8 N=19	208.1 Þ ± 5.9 N=18[b,d]	216.5 ± 5.3 N=34[c,d]
Relative Maternal Feed Consumption (gd 8 to 9) (g/kg/day)[a] #	255.3 ††† ± 17.1 ŸŸŸ N=21[b]	224.2 ± 10.3 N=19[c,d]	249.3 ± 14.0 N=16[b,c]	202.6 ÞÞ ± 5.9 N=20[b,d]	204.2 ÞÞ ± 4.0 N=33[b,c]
Relative Maternal Feed Consumption (gd 9 to 10) (g/kg/day)[a]	222.9 ‡‡‡ ± 8.4 §§ N=23	225.2 ± 8.7 N=21[b]	234.9 ± 7.6 N=18[b]	198.8 * ± 4.7 N=20[b,c]	204.8 ± 3.4 N=35[b]
Relative Maternal Feed Consumption (gd 10 to 11) (g/kg/day)[a]	218.8 ‡‡‡ ± 10.0 §§§ N=19[b]	229.9 ± 8.3 N=22	230.3 ± 6.9 N=18[b]	197.4 ± 6.4 N=20[d]	193.4 * ± 2.8 N=33[c,d]
Relative Maternal Feed Consumption (gd 11 to 12) (g/kg/day)[a]	228.1 ‡ ± 11.6 N=23	203.7 ± 5.1 N=21[b]	222.9 ± 9.0 N=18[b]	197.0 * ± 4.6 N=20[b,d]	230.6 ± 8.0 N=33[b,c,d]
Relative Maternal Feed Consumption (gd 12 to 13) (g/kg/day)[a]	198.3 ‡‡ ± 5.9 N=23	198.8 ± 6.0 N=21[c]	221.2 ± 7.5 N=19	192.0 ± 5.9 N=22	213.4 ± 5.6 N=34[c,d]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 5 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 13 to 14) (g/kg/day)[a]	191.7 ‡ ± 4.2 § N=23	195.8 ± 4.9 N=20[b,c]	205.9 ± 4.9 N=18[b]	189.8 ± 6.6 N=21[b]	209.4 * ± 4.1 N=36
Relative Maternal Feed Consumption (gd 14 to 15) (g/kg/day)[a]	182.2 ± 4.1 N=23	182.8 ± 4.9 N=21[b]	190.7 ± 5.8 N=19	177.8 ± 4.6 N=22	187.3 ± 5.0 N=36
Relative Maternal Feed Consumption (gd 15 to 16) (g/kg/day)[a]	171.5 ‡‡ ± 3.4 §§ N=23	179.0 ± 4.5 N=22	181.5 ± 6.1 N=19	162.0 ± 3.5 N=22	163.6 ± 2.6 N=35[c]
Relative Maternal Feed Consumption (gd 16 to 17) (g/kg/day)[a]	162.9 ± 4.7 N=23	160.3 ± 3.4 N=22	166.1 ± 3.4 N=19	161.2 ± 4.6 N=22	159.9 ± 2.4 N=35[c]
Relative Maternal Feed Consumption (gd 5 to 10) (g/kg/day)[a,e] #	235.6 † ± 12.1 Ÿ N=20[f]				209.5 Þ ± 3.7 N=28[f]
Relative Maternal Feed Consumption (gd 5 to 16) (g/kg/day)[a,g]	202.6 ± 5.7 N=17[f]	206.9 ± 5.3 N=14[f]	222.0 ± 6.7 N=12[f]	199.3 ± 7.0 N=17[f]	
Relative Maternal Feed Consumption (gd 10 to 17) (g/kg/day)[a,e]	189.6 ± 4.8 N=19[f]				194.5 ± 3.4 N=31[f]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 6 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 0 to 17) (g/kg/day)[a]					
	187.1 ± 4.9 N=14[f]	197.0 ± 5.8 N=13[f]	198.2 ± 7.0 N=8[f]	178.6 ± 3.8 N=14[f]	194.3 ± 5.1 N=20[f]

[a]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd = gestational day.

[b]Decrease in N is due to one or more feeders spilling, and therefore the feed weight was excluded.

[c]Decrease in N is due to the feed being contaminated for one or more animals, and therefore the feed weight was excluded.

[d]Decrease in N is due to the feed consumption value for one or more animals being a statistical outlier, and therefore they were excluded.

[e]This endpoint was only calculated for the 0 and 30,000 mg/m^3 dose groups.

[f]Decrease in N is due to interim feed consumption value(s) for one or more dams being missing, and therefore the overall feed consumption value could not be calculated.

[g]This endpoint was only calculated for the 0, 2000, 10,000, and 20,000 mg/m^3 dose groups.

#Levene's Test for homogeneity of variances was significant ($p<0.05$); therefore, robust regression methods were used to test all treatment effects.

†$p<0.05$; Wald Chi-square Test for overall treatment effect in robust regression model.

††$p<0.01$; Wald Chi-square Test for overall treatment effect in robust regression model.

†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.

Ÿ$p<0.05$; Linear trend test in robust regression model.

ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Þ$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.

ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.

ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

‡$p<0.05$; ANOVA Test.

‡‡$p<0.01$; ANOVA Test.

‡‡‡$p<0.001$; ANOVA Test.

§$p<0.05$; Test for Linear Trend.

§§$p<0.01$; Test for Linear Trend.

§§§$p<0.001$; Test for Linear Trend.

*$p<0.05$; Dunnett's Test.

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 1 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
ALL LITTERS[a]:	23	22	19	22	36
No. Corpora Lutea per Dam[b]	12.96 ± 0.38 N=23	12.18 ± 0.57 N=22	12.78 ± 0.36 N=18[c]	13.18 ± 0.56 N=22	13.19 ± 0.47 N=36
No. Implantation Sites per Litter[b]	12.74 ± 0.35 N=23	12.00 ± 0.61 N=22	12.68 ± 0.33 N=19	13.23 ± 0.46 N=22	12.89 ± 0.37 N=36
Percent Preimplantation Loss per Litter[b]	2.06 ± 0.93 N=23	5.99 ± 3.74 N=22	3.93 ± 1.52 N=18[c]	2.61 ± 0.99 N=22	4.81 ± 1.24 N=36
No. Resorptions per Litter[b]	0.61 ± 0.16 N=23	1.27 ± 0.52 N=22	0.74 ± 0.18 N=19	1.68 ± 0.86 N=22	1.56 ± 0.48 N=36
Percent Resorptions per Litter[b]	4.88 ± 1.31 N=23	11.03 ± 4.90 N=22	5.57 ± 1.40 N=19	12.91 ± 6.21 N=22	13.75 ± 4.52 N=36
No. Litters with Resorptions	11	9	10	10	20
% Litters with Resorptions	47.83	40.91	52.63	45.45	55.56

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 2 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Late Fetal Deaths per Litter[b]	0.13 ± 0.07 N=23	0.00 ± 0.00 N=22	0.11 ± 0.07 N=19	0.09 ± 0.06 N=22	0.03 ± 0.03 N=36
Percent Late Fetal Deaths per Litter[b]	1.02 ± 0.56 N=23	0.00 ± 0.00 N=22	0.70 ± 0.49 N=19	0.65 ± 0.45 N=22	0.20 ± 0.20 N=36
No. Litters with Late Fetal Deaths	3	0	2	2	1
% Litters with Late Fetal Deaths	13.04	0.00	10.53	9.09	2.78
No. Nonlive Implants per Litter[b,d]	0.74 ± 0.19 N=23	1.27 ± 0.52 N=22	0.84 ± 0.22 N=19	1.77 ± 0.86 N=22	1.58 ± 0.48 N=36
Percent Nonlive Implants per Litter[b,d]	5.90 ± 1.52 N=23	11.03 ± 4.90 N=22	6.28 ± 1.59 N=19	13.56 ± 6.16 N=22	13.95 ± 4.52 N=36
No. Litters with Nonlive Implants[d]	11	9	10	12	20
% Litters with Nonlive Implants[d]	47.83	40.91	52.63	54.55	55.56
No. Litters with 100% Nonlive Implants[d]	0	1	0	2	3
% Litters with 100% Nonlive Implants[d]	0.00	4.55	0.00	9.09	8.33

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 3 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Adversely Affected Implants per Litter[b,e]	0.83 ± 0.20 N=23	1.36 ± 0.52 N=22	0.89 ± 0.23 N=19	1.82 ± 0.85 N=22	1.78 ± 0.48 N=36
Percent Adversely Affected Implants per Litter[b,e]	6.69 ± 1.64 N=23	11.66 ± 4.85 N=22	6.63 ± 1.63 N=19	13.87 ± 6.15 N=22	15.38 ± 4.50 N=36
No. Litters with Adversely Affected Implants[e]	12	11	10	12	21
% Litters with Adversely Affected Implants[e]	52.17	50.00	52.63	54.55	58.33
LIVE LITTERS[f]:	23	21	19	20	33
No. Live Fetuses per Litter[b]	12.00 ± 0.39 N=23	11.24 ± 0.68 N=21	11.84 ± 0.27 N=19	12.60 ± 0.49 N=20	12.33 ± 0.26 N=33
Percent Male Fetuses per Litter[b]	50.03 ± 3.11 N=23	46.46 ± 3.82 N=21	52.95 ± 3.70 N=19	44.67 ± 3.14 N=20	45.58 ± 2.99 N=33
No. **Male** Fetuses per Litter[b]	6.09 ± 0.47 N=23	5.38 ± 0.45 N=21	6.21 ± 0.41 N=19	5.65 ± 0.48 N=20	5.61 ± 0.36 N=33

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 4 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. **Female** Fetuses per Litter[b]	5.91 ± 0.37 N=23	5.86 ± 0.52 N=21	5.63 ± 0.48 N=19	6.95 ± 0.47 N=20	6.73 ± 0.39 N=33
Average Fetal Body Weight (g) per Litter[b]	0.9994 ±0.0265 N=23	1.0114 ±0.0212 N=21	0.9471 ±0.0241 N=19	0.9492 ±0.0288 N=20	1.0248 ±0.0249 N=33
Average **Male** Fetal Body Weight (g) per Litter[b]	1.0114 ±0.0309 N=23	1.0088 ±0.0166 N=20[g]	0.9605 ±0.0254 N=19	0.9722 ±0.0311 N=20	1.0367 ±0.0261 N=33
Average **Female** Fetal Body Weight (g) per Litter[b]	0.9961 ±0.0246 N=23	0.9937 ±0.0227 N=21	0.9344 ±0.0231 N=19	0.9343 ±0.0281 N=20	1.0141 ±0.0239 N=33

[a]Includes all dams pregnant at terminal sacrifice on gestational day 17; litter size = no. implantation sites per dam.

[b]Reported as the mean ± S.E.M.

[c]Decrease in N is due to the right ovary for one female inadvertently being lost prior to the corpora lutea being counted.

[d]Nonlive = late fetal deaths plus resorptions.

[e]Adversely affected = nonlive plus malformed.

[f]Includes only dams with live fetuses; litter size = no. live fetuses per dam.

[g]Decrease in N is due to one litter having female fetuses only.

Table 6. Summary and Statistical Analysis of External Malformations and Variations (page 1 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Fetuses Examined[a]	276	236	225	252	407
No. Litters Examined[b]	23	21	19	20	33
No. Fetuses with External Malformations per Litter[c,d]	0.09 ± 0.06 N=23	0.10 ± 0.07 N=21	0.05 ± 0.05 N=19	0.05 ± 0.05 N=20	0.21 ± 0.11 N=33
No. **Male** Fetuses with External Malformations per Litter[c,d]	0.04 ± 0.04 N=23	0.05 ± 0.05 N=20	0.05 ± 0.05 N=19	0.00 ± 0.00 N=20	0.09 ± 0.05 N=33
No. **Female** Fetuses with External Malformations per Litter[c,d]	0.04 ± 0.04 N=23	0.05 ± 0.05 N=21	0.00 ± 0.00 N=19	0.05 ± 0.05 N=20	0.12 ± 0.07 N=33
Percent Fetuses with External Malformations per Litter[c,d]	0.85 ± 0.61 N=23	0.66 ± 0.46 N=21	0.38 ± 0.38 N=19	0.36 ± 0.36 N=20	1.64 ± 0.85 N=33
Percent **Male** Fetuses with External Malformations per Litter[c,d]	0.62 ± 0.62 N=23	0.83 ± 0.83 N=20	0.88 ± 0.88 N=19	0.00 ± 0.00 N=20	1.89 ± 1.16 N=33
Percent **Female** Fetuses with External Malformations per Litter[c,d]	0.72 ± 0.72 N=23	0.95 ± 0.95 N=21	0.00 ± 0.00 N=19	1.25 ± 1.25 N=20	1.29 ± 0.74 N=33

Table 6. Summary and Statistical Analysis of External Malformations and Variations (page 2 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Fetuses with External Malformations[d]	2	2	1	1	7
% Fetuses with External Malformations[d]	0.72	0.85	0.44	0.40	1.72
No. Litters with External Malformations[e]	2	2	1	1	4
% Litters with External Malformations[e]	8.70	9.52	5.26	5.00	12.12
No. Fetuses with External Variations per Litter[c,d]	0.13 ± 0.07 N=23	0.14 ± 0.10 N=21	0.11 ± 0.07 N=19	0.15 ± 0.08 N=20	0.00 ± 0.00 N=33
No. **Male** Fetuses with External Variations per Litter[c,d]	0.09 ± 0.06 N=23	0.05 ± 0.05 N=20	0.05 ± 0.05 N=19	0.05 ± 0.05 N=20	0.00 ± 0.00 N=33
No. **Female** Fetuses with External Variations per Litter[c,d]	0.04 ± 0.04 N=23	0.10 ± 0.10 N=21	0.05 ± 0.05 N=19	0.10 ± 0.07 N=20	0.00 ± 0.00 N=33
Percent Fetuses with External Variations per Litter[c,d]	1.34 ± 0.76 N=23	1.27 ± 0.99 N=21	0.81 ± 0.56 N=19	1.26 ± 0.69 N=20	0.00 ± 0.00 N=33
Percent **Male** Fetuses with External Variations per Litter[c,d]	1.45 ± 1.00 N=23	0.56 ± 0.56 N=20	0.66 ± 0.66 N=19	1.00 ± 1.00 N=20	0.00 ± 0.00 N=33

Table 6. Summary and Statistical Analysis of External Malformations and Variations (page 3 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Percent **Female** Fetuses with External Variations per Litter[c,d]	0.72 ± 0.72 N=23	1.59 ± 1.59 N=21	1.05 ± 1.05 N=19	1.39 ± 0.98 N=20	0.00 ± 0.00 N=33
No. Fetuses with External Variations[d]	3	3	2	3	0
% Fetuses with External Variations[d]	1.09	1.27	0.89	1.19	0.00
No. Litters with External Variations[e]	3	2	2	3	0
% Litters with External Variations[e]	13.04	9.52	10.53	15.00	0.00

[a]Only live fetuses were examined for malformations and variations.

[b]Includes only litters with live fetuses.

[c]Reported as the mean ± S.E.M.

[d]Fetuses with one or more malformations or variations.

[e]Litters with one or more fetuses with malformations or variations.

Table 7. Summary of Morphological Abnormalities in CD-1 Mouse Fetuses: Listing by Defect Type[a]
(page 1 of 1)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
EXTERNAL MALFORMATIONS					
Total No. of Fetuses Examined for External Malformations[b]	276	236	225	252	407
No. of Fetuses with External Malformations[c]	2	2	1	1	7
% Fetuses with External Malformations	0.7%	0.8%	0.4%	0.4%	1.7%
Total No. of Litters Examined for External Malformations[d]	23	21	19	20	33
No. of Litters with External Malformations[e]	2	2	1	1	4
% Litters with External Malformations	8.7%	9.5%	5.3%	5.0%	12.1%
Encephalocele		1(1)			
Cleft Palate	2(2)	1(1)	1(1)	1(1)	7(4)
Gastroschisis					1(1)
EXTERNAL VARIATIONS					
Total No. of Fetuses Examined for External Variations[b]	276	236	225	252	407
No. of Fetuses with External Variations[c]	3	3	2	3	0
% Fetuses with External Variations	1.1%	1.3%	0.9%	1.2%	0.0%
Total No. of Litters Examined for External Variations[d]	23	21	19	20	33
No. of Litters with External Variations[e]	3	2	2	3	0
% Litters with External Variations	13.0%	9.5%	10.5%	15.0%	0.0%
Abnormal Rugae in Midline of Palate			1(1)	1(1)	
Hematoma: Face		2(1)			
Hematoma: Head	2(2)	1(1)			
Hematoma: Neck	1(1)	1(1)	1(1)	2(2)	
Hematoma: Shoulder	1(1)				

[a]A single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).

[b]Only live fetuses were included.

[c]Fetuses with one or more malformations/variations.

[d]Includes only litters with live fetuses.

[e]Litters with one or more malformed/variant fetuses.

Quality Assurance Statement

Draft Final Report

TITLE: Range-Finding Tolerance Study for the Developmental Toxicity Evaluation of Inhaled Gasoline Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

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STUDY INITIATION DATE: August 30, 2004

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Table of Contents

SUMMARY

The purpose of this study was to provide maternal and developmental toxicity data relative to a 6-day exposure regimen of inhaled gasoline methyl tertiary butyl ether (MTBE) vapor condensate during early organogenesis in gravid mice. These data were used to determine the tolerance of the dams and conceptuses to the highest target exposure concentration of 30,000 mg/m^3 to be possibly selected for the definitive toxicity test. A developmental toxicity evaluation of Gasoline MTBE Vapor Condensate by inhalation to mice was mandated by API 211(b) Vapor Condensate Test Program (U.S. EPA, 1994) to evaluate a series of fuel additives for reproductive and developmental toxicity in animal models. One of the developmental studies involved whole-body inhalation exposure of timed-pregnant CD-1® mice for at least 6 hours/day to baseline gasoline vapor condensate with 21.5% MTBE at target concentrations of 0, 2000, 10,000, and 20,000 mg/m^3 (the last is 50% of the lower explosive limit). This study was conducted to confirm and extend the findings observed in that earlier study.

RTI International (RTI; Research Triangle Park, NC) was responsible for study design, protocol generation, setting up mating and assignment of plug-positive study females, necropsy of the maternal and fetal animals on gestational day (gd) 17, generation of summary and individual data tables, and study draft and final reports (with RTI QA oversight). RTI's Quality Assurance Unit performed a prestudy on-site inspection, reviewed the protocol and any amendments, and monitored all phases of the study in which RTI personnel participated. Huntingdon Life Sciences (HLS; East Millstone, NJ) was responsible for receipt of the test material, prestudy and study generation and analyses of the test vapors, receipt, quarantine, and housing of the test females and breeder males, setting up the mating and assignment of the study females, in-life observations, loading and unloading study females into and out of chambers, and submission of interim and final inhalation reports. The Quality Assurance Unit of HLS reviewed the protocol and monitored the facilities, equipment, personnel, methods, practices, records, raw data, draft and final inhalation reports, and controls used in this study to assure that they were in conformance with company standard operating procedures and the referenced Good Laboratory Practice (GLP) regulations.

This study was conducted in accordance with 79.61, CFR Vol. 59, No. 122, 27 June 1994 (U.S. EPA, 1994) and performed according to the protocol and HLS' and RTI's SOPs. This study complied with all appropriate parts of the USDA Animal Welfare Act regulations: 9 CFR

Parts 1 and 2 Final Rules, *Federal Register*, Vol. 54, No. 168, August 31, 1989, pp. 36112-36163, effective October 30, 1989, and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 55, No. 32, February 15, 1991, pp. 6426-6505, effective March 18, 1991, and U.S. EPA TSCA GLPs (U.S. EPA, 1989).

MATERIALS AND METHODS

Test Material and Dose Formulations

The test material, Gasoline MTBE Vapor Condensate (MRD-00-713; "API 211BG with MTBE Vapor Condensate") was a colorless liquid and identified by the supplier(Chevron Global Technology Services Company, Richmond, CA) as Lot/Batch Number API 00-02. Information on identity, strength, purity, and composition of Gasoline MTBE Vapor Condensate was provided by the Sponsor and documented in the raw data and in this final report (Appendix IV, protocol attachment). Methods of synthesis, fabrication, or derivation were documented by the Sponsor and located at API. The test material was stable and stored under ambient conditions in an outside solvent shed except when in use in the inhalation laboratory. The test substance was handled as a flammable liquid. Detailed information on chemical handling is provided in the MSDS attached to the protocol (Appendix IV).

Animals and Husbandry

The proposed test animals were Caesarean-originated Virus Antibody Free (VAF) Crl:CD-1® (ICR) BR outbred albino mice supplied by Charles River Laboratories, Inc., Raleigh, NC. The use of live animals was requested by the Sponsor and required by U.S. EPA OPPTS Testing Guidelines (U.S. EPA, 1998). Alternative test systems are not available for the assessment of chemical effects on prenatal mammalian development. The Charles River CD-1® mouse has been the subject of choice on developmental toxicology contracts at RTI since 1976. Large historical databases for reproductive performance and prevalence of spontaneous malformations in control mice are available from studies conducted at RTI (currently based on over 348 control litters).

The actual dates of all major phases of the study are presented in Table A.

Table A. Study Schedule

Event	Dates
Animals arrive at HLS:	August 31, 2004
Quarantine (14 days):	August 31 – September 14, 2004
Animals paired:	September 16-18, 2004
Dates of gd 0:	September 17-19, 2004
TSCA experimental start date:	September 22, 2004
Exposure dates (gd 5 through 10):	September 22-29, 2004
Scheduled termination (gd 17)	October 4-6, 2004
TSCA experimental termination date:	October 6, 2004
Submission of draft data on test atmospheres to Sponsor:	October 6, 2004 (within 1 week after the last exposure date, September 29, 2004

Fifty (50) nulliparous female mice were ordered for this range-finding study. One hundred (100) male mice, 9-11 weeks old, and of the same strain and from the same supplier, were also ordered. One extra female was received with the shipment. One female that would have been over the designated weight range on gestational day (gd) 0 (35 g) was excluded prior to mating. Fifty (50) male mice were used to generate timed-mated animals for this study, and all 100 of the males were used to generate timed-mated animals for the subsequent definitive developmental toxicity study which will require the mating of 170 female mice to generate 140 plug-positive females. Acclimating all 100 male mice assured that they were the same age for this range-finding study and subsequent definitive study, and have been exposed to the same environmental conditions, etc. Female mice were 7-9 weeks old at arrival and were 9-11 weeks of age and 20-35 g in weight on gd 0. Fifty (50) females were required to generate 20 plug-positive females in 2 to 3 consecutive days; 20 plug-positive females (10 per group and 2 groups) were required to supply the minimum number (based on EPA's guidance; e.g., OPPTS 870.3600; U.S. EPA, 1996; for inhalation developmental toxicity studies) of pregnant animals to assess the maternal and embryo/fetal tolerance of the highest concentration.

During an approximately 14-day quarantine and acclimation period at the HLS testing facility, animals were checked for viability twice daily. Prior to study assignment, all animals were examined to ascertain suitability for study. The HLS veterinarian formally released these animals for use by signature and date. Males and females were individually housed in stainless

steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female were housed together. During cohabitation, male and female mice were housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). Each cage was fitted to secure a glass feeder jar with a stainless steel lid. Clean feed jars and fresh feed were provided at least weekly for periods when feed consumption was not being recorded and at each interval when feed consumption was recorded. After cessation of exposures began on gd 11, a stainless steel, perforated insert was placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. Feed (PMI 5002 Certified Meal) was available *ad libitum*, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer were maintained on file at the HLS testing facility, and there were no known contaminants found in the feed. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) was available *ad libitum* via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses were conducted by Elizabethtown Water Company to assure that water met standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS testing facility. There were no known contaminants that interfered with the objectives of this study. At all times, animals were housed, handled, and used according to the National Research Council Guide (NRC, 1996).

A 12-hour light/dark cycle was provided via automatic timer. Temperature and relative humidity were monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18 to 26°C for temperature and 30 to 70% relative humidity was maintained to the maximum extent possible (NRC, 1996).

Each animal was assigned a temporary identification number (designated on each cage) upon receipt. During the second week of the quarantine/acclimation period, the 51 females received were tail tattooed with consecutive numbers, 1 through 51. The 100 males were also tail tattooed with consecutive numbers, 1 through 100. After selection for use on the study, mating, indication of copulation, and assignment to either of the 2 groups, each female was ear tagged with a number assigned by the HLS testing facility. This number, plus the study number, comprised the unique animal number for each animal. Each cage was provided with a cage card

that was color coded for exposure level identification and contained the study and animal numbers.

Some adult toxicity (e.g., narcosis) was caused by exposure to the high concentration. It was anticipated that the concentration employed would not result in irritation or corrosion to the respiratory tract of the test animals. Animals were not subjected to undue pain or distress. All procedures used in this study were designed to avoid discomfort, distress, and pain to the animals. The HLS IACUC Protocol Review Subcommittee and the RTI IACUC reviewed the protocol and found it to be in compliance with appropriate animal welfare regulations.

Immediately prior to pairing, each female was weighed and subjected to a clinical examination. For breeding, 1 male with 1 female pairing was employed since other pairing patterns (e.g., 1 male with 2 females) may have resulted in an unacceptable number of plug-positive, nonpregnant females and/or sire effects. Individual females were placed in polycarbonate "shoebox" cages with stainless steel lids with singly-housed males. On the following morning and each morning thereafter, the females were examined for the presence of a vaginal or dropped copulation plug (Hafez, 1970). The day on which copulation plugs were found was designated as gd 0. Plug-positive females (dams) were individually housed until scheduled sacrifice on gd 17. Plug-negative females were retained in the same male's cage and checked for plugs on successive mornings until insemination occurred or the treatment groups were filled, whichever came first. RTI staff were present during the initial day of mating (September 17, 2004) to confirm expertise of HLS staff to detect vaginal copulation plugs and then set up the exposure schedule, based on the gd 0 dates. HLS staff continued to evaluate females for vaginal copulation plugs until both groups were filled and then completed the exposure schedule. When all treatment groups were filled, the remaining plug-negative and plug-positive females were sacrificed by asphyxiation with CO_2. The males were retained for the definitive developmental toxicity study. The fate of all animals was fully documented.

Study Design

This preliminary study was conducted with 1 treatment group and 1 vehicle control group, each comprised of 10 plug-positive female mice (Table B).

Table B. Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	10	6	5 through 10	0
2	10	6	5 through 10	30,000

The 30,000 mg/m^3 concentration was chosen to determine the tolerance of the dams and conceptuses to the highest exposure concentration to be possibly selected for the definitive toxicity study.

The test substance was administered as a vapor in the breathing air of the animals. The test atmosphere was generated by an appropriate procedure determined during prestudy trials. The trials were performed (at least two 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform conditions throughout the exposure chambers. The whole-body exposure chambers each had a volume of approximately 1000 liters. The chambers were operated at a minimum flow rate of 200 liters per minute. The final airflow was set to provide at least 1 air change in 5 minutes (12 air changes/hour) and a T_{99} equilibrium time of at most 23 minutes. This chamber size and airflow rate was considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of each daily 6-hour exposure, all animals remained in the chamber for a minimum of the T_{99} equilibrium time. During this time, the chamber was operated at approximately the same flow rate using clean air only.

A nominal exposure concentration was calculated. The flow of air through the chamber was monitored using appropriate calibrated equipment. The test substance consumed during the exposure was divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each 6-hour exposure, measurements of airborne concentrations were performed in the animals' breathing zone at least 4 times using an appropriate sampling procedure and IR analytical procedure. Airborne test material concentrations were within +/- 10% of the target concentration. One sample per chamber during the trials period and the treatment period was analyzed by gas chromatography to characterize at least 10 major components (comprising at least 80% by weight of the test substance) to show test substance stability and comparison between the neat liquid test substance and the vaporized test atmospheres. During the treatment period, particle

size determinations were performed once per chamber using a TSI Aerodynamic Particle Sizer to confirm the absence of particulate test substance condensate in the exposure atmosphere.

Chamber temperature, humidity, airflow rate, and static pressure were monitored continuously and recorded every 30 minutes during exposure. Chamber temperature and relative humidity were maintained, to the maximum extent possible, between 20 to 24°C and 40 to 60%, respectively. Chamber oxygen levels (maintained at least 19%) were measured pretest and at the beginning, middle, and end of the study. Air samples were taken in the vapor generation area pretest and at the beginning, middle, and end of the study. Light (maintained approximately 30 foot-candles at 1.0 meter above the floor) and noise levels (maintained below 85 decibels) in the exposure room were measured pretest and at the beginning, middle, and end of the study.

The minimum frequency of chamber activity during the treatment period is summarized below:

Activity	Frequency/Chamber
Measured test substance concentration	4X/day
Measured test substance characterization	1X
Particle size	1X
Temperature	13X/day
Relative humidity	13X/day
Airflow rate	13X/day
Static pressure	13X/day
Nominal test substance concentration (excluding the air control chamber)	1X/day
Rotation pattern of exposure cages	1X/day
Loading/unloading verification	1X/day

Plug-positive female mice (dams) were assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights between dose groups on gd 0. Females were exposed to Gasoline MTBE Vapor Condensate or air 6 hours per day from gd 5 through 10. For each daily exposure, females were transferred to inhalation cages, and the cages were moved into the appropriate chambers for exposure. Following each daily exposure, females were transferred back to home caging for feed consumption measurements overnight.

Clinical observations of all animals were made once daily on gd 0 through 4 (prior to exposure period) and on gd 11 through 17 (prior to necropsy) and twice daily, prior to and immediately after each daily exposure, throughout the exposure period (gd 5 through 10). In

addition, during each daily exposure period, animals were observed at least once during each exposure. This was routinely performed near the middle of each exposure.

Dams were weighed in the mornings (prior to exposures for those days that exposures occurred) on gd 0, 5, 6, 7, 8, 9, 10, 12, 14, 16, and 17. Maternal weight gains were calculated for gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-12, 12-14, 14-16, 16-17, 5 through 10 (exposure period), 10 through 17 (postexposure period), and 0 through 17 (gestational period).

Maternal feed consumption was evaluated in the mornings from gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-12, 12-14, 14-16, 16-17, 5 through 10 (exposure period), 10 through 17 (postexposure period), and 0 through 17 (gestation period).

No maternal animals died during the course of the study. On gd 17, approximately 1 to 1½ days before expected parturition, all surviving maternal animals were killed by CO_2 asphyxiation by RTI staff. The thoracic and abdominal cavities and organs were examined, and pregnancy status was confirmed by uterine examination. Uteri that presented no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites that may have undergone very early resorption (Salewski, 1964). At sacrifice, the body, liver, uterus, paired adrenal glands, and paired kidneys of each plug-positive female were weighed. Ovarian corpora lutea were counted and uterine contents (i.e., number of implantation sites, early and late resorptions, dead fetuses, live fetuses) recorded.

Live fetuses were counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each fetus was killed by intraperitoneal injection of sodium pentobarbital, dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcass was blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses were destroyed by incineration.

Statistics

The unit of comparison was the dam or the litter, as appropriate. The single treatment group was compared by RTI staff to the concurrent control group using the Student's *t*-Test (SAS® Releases 8.0 and 8.2; SAS® Institute 1999a, b, c, d, e; 2000; 2001). Litter derived

percentage data were arcsine transformed and then analyzed using ANOVA (Analysis of Variance) (SAS® Institute 1999a, b, c, d, e; 2000; 2001) weighted according to litter size.

Storage of Records

All data documenting experimental details and study procedures and observations were recorded and maintained as raw data. At the completion of the study, all reports, raw data, preserved specimens, and retained samples will be maintained in RTI's secure archives for a period of 1 year after submission of the signed final report. The Sponsor will be contacted in order to determine the final disposition of these materials.

Personnel

This study was conducted at HLS (Mr. G.M. Hoffman, Principal Investigator; Animal Research Facility Veterinarian, Dr. Teresa S. Kusznir; Animal Research Facility Director, Mr. I. Vanterpool; Necropsy Laboratory Supervisor, Ms. G.E. Baxter; Inhalation Laboratory Supervisor, Mr. S. Cracknell; Associate Director of Formulation Chemistry Services, Ms. K. Saladdin; Quality Assurance, Ms. N.S. Iacono) under contract to the American Petroleum Institute (API; Mr. T.M. Gray, Sponsor's Representative). Dr. R.W. Tyl of RTI served as Study Director. RTI Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), and Mr. W.P. Ross (Neurotoxicology Supervisor). RTI Quality Assurance personnel were Ms. D.A. Drissel (Manager), Ms. C.A. Ingalls, Ms. S.C. Wade, and Ms. M.M. Oh.

The final report was prepared by Dr. R.W. Tyl, Ms. M.C. Marr, and Ms. C.A. Winkie, with assistance from Ms. C.B. Myers for statistical analyses and generation of tables, and by Mr. T.W. Wiley for data entry. Ms. M.C. Marr was responsible for all transfer of custody procedures for fetal viscera, fetal carcasses, data generated at HLS by HLS staff from HLS to RTI, and for archiving the study records.

RESULTS

Test Chamber Generation and Analyses

The results of the chamber generation and analyses are presented in Table 1 and Appendix I. In the control chamber (0.0 mg/m^3), there was no detectable test material (estimated LOQ [limit of quantification] is 433 mg/m^3). The size (mass median aerodynamic diameter; MMAD) of the particulates was approximately 1.3 μm, with the geometric standard deviation (GSD) 2.303. The total mass concentration (TMC) was 4.41 x 10^{-3} mg/m^3. Mean temperature was 21°C, and mean relative humidity was 46.6%. In the chamber with the test material and target concentration (30,000 mg/m^3), the mean analytical concentration was 30,688 mg/m^3 (102.3% of target). The size (MMAD) of the particulate was approximately 3.667 μm, with the GSD 2.315. The TMC was 1.02 x 10^{-2} mg/m^3. Mean temperature was 22°C, and relative humidity was 46.6%. The analytical profile of the test material (in Appendix I) indicated approximately 26.85% MTBE, 31.06% isopentane, and 8.69% n-butane. All remaining hydrocarbon concentrations were < 5%. The MTBE concentration (26.85%) was slightly higher than the initial analytical profile (included in the information from the supplier) when it was approximately 21.5%. This was due to co-elution of MTBE with 2,3-dimethylbutane (Appendix I).

Maternal Toxicity

Of the 10 plug-positive females per group and 2 groups (0 and 30,000 mg/m^3), no females were removed from study, died, or were euthanized moribund on study. One female in each group was nonpregnant, so the pregnancy rate was 90% in both groups. Two litters at 0 mg/m^3 and 1 litter at 30,000 mg/m^3 were fully resorbed, so the numbers and percent of live litters were 7 of 9 (77.8%) at 0 mg/m^3 and 8 of 9 (88.9%) at 30,000 mg/m^3. Overall, the pregnancy rate was 90.0% (18/20) and the percent with live litters was 75% (15/20). Maternal body weights were statistically equivalent between the 2 groups throughout gestation, on gd 0, 5-10, 12, 14, 16, and 17 for in-life, and on gd 17 at sacrifice. Maternal body weight change was similarly unaffected between the 2 groups throughout gestation, including the intervals gd 0-5 (pre-exposure period); 5-6, 6-7, 7-8, 8-9, 9-10, 10-12, 12-14, 14-16, 16-17, and 5-10 (exposure period); gd 10-17 (postexposure period); gd 0-17 (gestation); and corrected gestational body weight change (gd 0-17 minus gravid interim weight) (Table 2).

At scheduled necropsy on gd 17, the absolute weights of the gravid uterus and maternal liver, paired adrenal glands, and paired kidneys were equivalent between the 2 groups. Relative weights (as a percent of the sacrifice weight) for the specified organs were also equivalent between the 2 groups (Table 2).

There were no maternal clinical signs before or after the exposure period (gd 5 through 10) or before or after each daily 6-hour exposure period (Table 3).

Observations taken during each daily exposure period indicated "most" (defined by HLS as 51-99% of the animals exhibiting a given observation) of the females at 30,000 mg/m^3 exhibited "lethargy" on the first exposure day (gd 5), with the approximate incidence dropping to "some" on exposure days 2 through 6 (defined by HLS as 21-50% of the animals exhibiting a given observation), and then "few" observed on exposure days 7 and 8 (defined by HLS as <20% of the animals exhibiting a given observation) as the exposure period progressed.

Maternal feed consumption in g/day and g/kg body weight/day was equivalent across both groups for all intervals during gestation, although the mean consumption values at 30,000 mg/m^3 in g/day and g/kg/day were slightly (but not statistically significantly) lower for gd 5 through gd 9 during the exposure period (Table 4).

Uterine and Embryofetal Findings

Of the 9 dams pregnant in each group, there were no differences between groups for the number of ovarian corpora lutea per dam (a measure of eggs ovulated), uterine implantation sites per litter (a measure of zygotes implanted), or percent preimplantation loss/litter (the difference between ovarian corpora lutea and uterine implant sites). The number and percentage of resorptions/litter were clearly increased at 0 mg/m^3 due to 2 females with full litter resorption at 0 mg/m^3 and only 1 female at 30,000 mg/m^3 with full litter resorption. This difference was not statistically significantly different due to large variance terms in these parameters for both groups. All 9 litters at 0 mg/m^3 had 1 or more resorptions, and 4 of 9 litters at 30,000 mg/m^3 had 1 or more resorptions. The percentage of litters with resorptions was therefore significantly reduced at 30,000 mg/m^3. The number and percentage of late fetal deaths per litter were equivalent between the 2 groups. The number and percentage of litters with late fetal deaths were statistically equivalent between the 2 groups. The number and percentage of nonlive (resorptions plus late fetal deaths) implants per litter were statistically equivalent but clearly

higher at 0 mg/m^3 due to the greater number of resorptions and fully resorbed litters. The number and percentage of litters with 1 or more nonlive implants were significantly lower at 30,000 mg/m^3 due to fewer resorptions and fully resorbed litters. The number and percentage of adversely affected (nonlive plus malformed) implants and litters with 1 or more adversely affected implants were clearly reduced at 30,000 mg/m^3, but not statistically significantly, due to large variance terms (Table 5).

For the 7 live litters at 0 mg/m^3 and the 8 live litters at 30,000 mg/m^3, the number of live fetuses per litter, the percentage of male fetuses per litter, and the number of male and female fetuses per litter were all equivalent between the 2 groups. In addition, fetal body weight/litter, with sexes combined or separately, was also equivalent between the 2 groups (Table 5).

There were 71 live fetuses examined in the 7 litters at 0 mg/m^3 and 94 fetuses in the 8 litters at 30,000 mg/m^3. There was 1 fetus (in 1 litter) in each group with an external malformation (cleft palate), with no fetuses in either group with an external variation (Table 6). The fetal external malformation type and incidence are presented in Table 7. There were clearly no differences in fetal findings between the 2 groups.

DISCUSSION

It is clear that the maternal mice can easily tolerate 30,000 mg/m^3 of gasoline with MTBE vapor condensate. There were no deaths or moribund animals, no clinical signs before or after exposures, and only "lethargy" during the exposures, with the incidence high initially and dropping slowly over time. This lethargy (narcosis) was anticipated since it was observed in CD-1® female mice exposed to pure MTBE vapor at 7000 ppm only during the exposure periods (Bevan et al., 1997a).

It is also clear that embryofetal survival, growth, and development were also unaffected during the 6-day exposure period (gd 5 through 10) at 30,000 mg/m^3. The significant reduction in resorptions at 30,000 mg/m^3 is most likely due to the 2 litters fully resorbed at 0 mg/m^3 versus 1 litter at 30,000 mg/m^3, as well as all 9 litters at 0 mg/m^3 with at least 1 resorption, while only 4 of 9 litters at 30,000 mg/m^3 bore 1 or more resorptions. There were also 1 litter at 0 mg/m^3 and 2 litters at 30,000 mg/m^3 with at least 1 late fetal death. These effects may be due to the small number of litters per group, but they clearly indicate no risk to embryofetal survival, growth, or development at 30,000 mg/m^3.

The only fetal external malformation observed in this study was cleft palate, observed in 1 fetus each at 0 and 30,000 mg/m^3. This observed malformation cannot be due to the exposure since the exposures ceased at the end of gd 10, and cleft palate is induced at the time of palatal shelf fusion, which takes place on gd 14-15 in the mouse (Rugh, 1968) when exposures had already ceased. Cleft palate is likely a spontaneous malformation, as it is observed in RTI's historical control database for CD-1® mouse developmental toxicity studies.

Since cleft palate was observed in 1 fetus in the original ExxonMobil Biomedical Sciences, Inc. (EMBSI) study (2002) at 20,000 mg/m^3 and at a significantly increased incidence in the MTBE study at 7000 ppm (Bevan et al., 1997a), it is highly likely that cleft palate would occur at 30,000 mg/m^3 if the exposures continued through gd 16. Bevan et al. (1997b) reported increased adrenal gland weights in the rat MTBE multigeneration reproductive toxicity study. A mechanism for the fetal cleft palates was therefore proposed, whereby the maternal mice became highly stressed as they fought against the narcosis during exposures. This stress elevated corticosteroid production in and release from the maternal adrenal glands. Increased endogenous (or exogenous) corticosteroids in pregnant mice are known to result in cleft palate in their offspring. In the developmental toxicity study of MTBE in mice (Bevan et al., 1997a), maternal

adrenal glands were not weighed. They were weighed in the rat multigeneration MTBE study (Bevan et al., 1997b) and exhibited a concentration-related increase. Therefore, maternal adrenal weights were collected in this study with a very small "n" per group and a short exposure period. There were no differences between groups for absolute or relative maternal adrenal gland weights on gd 17 necropsy. It will be informative to see if adrenal gland weights are affected at 2000-20,000 mg/m^3 after 12 days of exposure, gd 5 through 16, and necropsy on gd 17.

The last finding (or lack thereof) in this study is the absence of any fetal external malformations (except for cleft palate; see above). There was no evidence of any effects on ventral midline closure. Although this was a small study, there were 71 fetuses examined at 0 mg/m^3 and 94 fetuses examined at 30,000 mg/m^3 (a concentration higher than those employed in the 2002 EMBSI study). Although the results of this range-finding study are not definitive, they do lead credence to the tentative conclusion that the low incidences of ectopia cordis and gastroschisis observed at 2000 and 10,000 (but not 20,000) mg/m^3 in the EMBSI study were not treatment or concentration related. The only differences between that study and this study are the source of the CD-1® mice (Charles River, Portage, MI, at EMBSI and Charles River, Raleigh, NC, in the present study), duration of exposure (EMBSI employed gd 5 through 17 with necropsy on gd 18, the present study employed gd 5 through 10 with necropsy on gd 17), the number of dams/group (25 in the EMBSI study and 10 in the present study), and the exposure concentration (EBMSI employed 4 groups [0, 2000, 10,000, and 20,000 mg/m^3] and the present study had 2 groups [0 and 30,000 mg/m^3]). Since the fetal malformations of concern are both failures of ventral midline body wall fusion, which takes place on gd 7-9 in the mouse (Rugh, 1968), the different durations are not germane since both studies employed exposure periods that encompassed the period of time when ventral midline body wall fusion occurs in the mouse.

Based on the results of this range-finding study, the definitive study should proceed with 25 plug-positive females/group at 0, 2000, 10,000, and 20,000 mg/m^3, with exposures on gd 5 through 16, and 40 plug-positive females at 30,000 mg/m3, with exposures on gd 5 through 10 and scheduled necropsy on gd 17.

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Table 1. Chamber Monitoring Results (page 1 of 1)

Target Chamber Concentrations:	0 mg/m^3	30,000 mg/m^3
Mean analytical chamber concentrations of gasoline MTBE vapor condensate (mg/m^3)[a]	0.00 ± 0.00	30,688 ± 1,595
Percent of target concentration	NA	102.29%
Particle Size Determinations:[b]		
MMAD (μm)	1.317	3.667
GSD	2.303	2.315
TMC (mg/m^3)	4.41×10^{-3}	1.02×10^{-2}
Temperature (°C)[c]	21.0 ± 0.0	22.0 ± 0.0
Relative humidity (%)[c]	46.6 ± 1.1	46.6 ± 0.7

[a] Mean of 4 assays measured by infrared spectroscopy

[b] Measured once

[c] Measured 13 times

MMAD = mass median aerodynamic diameter

GSD = geometric standard deviation

TMC = total mass concentration (measure of aerosol concentration)

NA = not applicable

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 1 of 4)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
SUBJECTS (No. Dams):		
No. on Study	10	10
No. Removed	0	0
No. Dead or Euthanized	0	0
No. Nonpregnant	1	1
No. (%) Pregnant at Scheduled Sacrifice	9 (90.0)	9 (90.0)
No. (%) with 100% Resorptions	2 (22.2)	1 (11.1)
Maternal Body Weight (gd 0) (g)[a]	26.6 ± 0.5 N=9	26.9 ± 0.4 N=9
Maternal Body Weight (gd 5) (g)[a]	27.8 ± 0.7 N=9	27.9 ± 0.3 N=9
Maternal Body Weight (gd 6) (g)[a]	28.3 ± 0.8 N=9	28.1 ± 0.4 N=9
Maternal Body Weight (gd 7) (g)[a]	28.8 ± 0.7 N=9	28.5 ± 0.4 N=9
Maternal Body Weight (gd 8) (g)[a]	29.4 ± 0.8 N=9	29.0 ± 0.4 N=9
Maternal Body Weight (gd 9) (g)[a]	29.7 ± 0.9 N=9	29.3 ± 0.5 N=9
Maternal Body Weight (gd 10) (g)[a]	30.4 ± 1.1 N=9	30.1 ± 0.5 N=9
Maternal Body Weight (gd 12) (g)[a]	33.0 ± 1.5 N=9	33.2 ± 0.9 N=9
Maternal Body Weight (gd 14) (g)[a]	36.2 ± 2.1 N=9	37.5 ± 1.3 N=9

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 2 of 4)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Maternal Body Weight (gd 16) (g)[a]	40.9 ± 2.9 N=9	43.4 ± 2.1 N=9
Maternal Body Weight (gd 17) (g)[a]	43.0 ± 3.3 N=9	46.1 ± 2.4 N=9
Maternal Body Weight (gd 17 at sacrifice) (g)[a]	42.11 ± 3.21 N=9	45.42 ± 2.40 N=9
Maternal Body Weight Change (gd 0 to 5) (g)[a]	1.2 ± 0.5 N=9	1.0 ± 0.2 N=9
Maternal Body Weight Change (gd 5 to 6) (g)[a]	0.5 ± 0.1 N=9	0.1 ± 0.2 N=9
Maternal Body Weight Change (gd 6 to 7) (g)[a]	0.5 ± 0.1 N=9	0.4 ± 0.1 N=9
Maternal Body Weight Change (gd 7 to 8) (g)[a]	0.5 ± 0.2 N=9	0.5 ± 0.2 N=9
Maternal Body Weight Change (gd 8 to 9) (g)[a]	0.4 ± 0.2 N=9	0.3 ± 0.1 N=9
Maternal Body Weight Change (gd 9 to 10) (g)[a]	0.7 ± 0.2 N=9	0.8 ± 0.2 N=9
Maternal Body Weight Change (gd 10 to 12) (g)[a]	2.5 ± 0.5 N=9	3.2 ± 0.5 N=9

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 3 of 4)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Maternal Body Weight Change (gd 12 to 14) (g)[a]	3.2 ± 0.7 N=9	4.3 ± 0.5 N=9
Maternal Body Weight Change (gd 14 to 16) (g)[a]	4.7 ± 0.8 N=9	5.9 ± 0.8 N=9
Maternal Body Weight Change (gd 16 to 17) (g)[a]	2.0 ± 0.4 N=9	2.7 ± 0.4 N=9
Maternal Body Weight Change (gd 5 to 10) (g)[a]	2.7 ± 0.5 N=9	2.2 ± 0.4 N=9
Maternal Body Weight Change (gd 10 to 17) (g)[a]	12.5 ± 2.3 N=9	16.0 ± 2.0 N=9
Maternal Body Weight Change (gestation) (g)[a]	15.5 ± 3.0 N=9	18.5 ± 2.2 N=9
Maternal Body Weight Change (corrected) (g)[a,b]	3.58 ± 0.91 N=9	2.95 ± 0.42 N=9
Gravid Uterine Weight (g)[a]	11.9289 ± 2.3183 N=9	15.5566 ± 2.0903 N=9
Maternal Liver Weight (g)[a]	2.2892 ±0.1507 N=9	2.3848 ±0.0963 N=9
Maternal Paired Adrenal Gland Weight (g)[a]	0.0116 ±0.0008 N=9	0.0103 ±0.0011 N=9

Table 2. Summary and Statistical Analysis of the Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 4 of 4)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Maternal Paired Kidney Weight (g)[a]		
	0.4221 ±0.0194 N=9	0.4303 ±0.0130 N=9
Relative Maternal Liver Weight (% sacrifice weight)[a]		
	5.4895 ± 0.1371 N=9	5.2984 ± 0.1390 N=9
Relative Maternal Paired Adrenal Gland Weight (% sacrifice weight)[a]		
	0.0283 ± 0.0017 N=9	0.0234 ± 0.0028 N=9
Relative Maternal Paired Kidney Weight (% sacrifice weight)[a]		
	1.0364 ± 0.0630 N=9	0.9770 ± 0.0786 N=9

[a]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd=gestational day.

[b]Weight change during gestation (gestational day 17 sacrifice weight minus gestational day 0 weight) minus gravid uterine weight.

Table 3. Summary of the Maternal Clinical Observations (page 1 of 1)

Observation	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Daily or Prior to Treatment:[a]		
Normal	10	10
Post Treatment:[b]		
Normal	10	10

[a]The daily clinical observations were recorded for gestational days 0 through 17 and on the days of treatment (gestational days 5 through 10) were recorded prior to treatment.

[b]These clinical observations were recorded for gestational days 5 through 10 only.

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 1 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
No. of Pregnant Dams	9	9
Maternal Feed Consumption (gd 0 to 5) (g/day)[a]	5.4 ± 0.3 N=7[b]	5.4 ± 0.3 N=8[b]
Maternal Feed Consumption (gd 5 to 6) (g/day)[a]	6.6 ± 1.1 N=9	5.2 ± 0.2 N=8[b]
Maternal Feed Consumption (gd 6 to 7) (g/day)[a]	6.1 ± 0.3 N=8[b]	5.5 ± 0.2 N=9
Maternal Feed Consumption (gd 7 to 8) (g/day)[a]	6.2 ± 0.6 N=7[b]	5.5 ± 0.2 N=9
Maternal Feed Consumption (gd 8 to 9) (g/day)[a]	6.5 ± 0.6 N=9	5.7 ± 0.1 N=9
Maternal Feed Consumption (gd 9 to 10) (g/day)[a]	5.7 ± 0.3 N=8[b]	5.6 ± 0.2 N=9
Maternal Feed Consumption (gd 10 to 12) (g/day)[a]	5.9 ± 0.4 N=9	5.9 ± 0.2 N=9
Maternal Feed Consumption (gd 12 to 14) (g/day)[a]	6.6 ± 0.6 N=9	6.9 ± 0.3 N=9
Maternal Feed Consumption (gd 14 to 16) (g/day)[a]	7.2 ± 0.7 N=8[c]	7.3 ± 0.3 N=9
Maternal Feed Consumption (gd 16 to 17) (g/day)[a]	7.1 ± 0.5 N=9	7.3 ± 0.4 N=9

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 2 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Maternal Feed Consumption (gd 5 to 10) (g/day)[a]	5.8 ± 0.4 N=7[d]	5.4 ± 0.1 N=8[d]
Maternal Feed Consumption (gd 10 to 17) (g/day)[a]	6.6 ± 0.5 N=8[d]	6.8 ± 0.3 N=9
Maternal Feed Consumption (gd 0 to 17) (g/day)[a]	5.5 ± 0.5 N=5[d]	5.8 ± 0.2 N=7[d]
Relative Maternal Feed Consumption (gd 0 to 5) (g/kg/day)[a]	198.3 ± 9.0 N=7[b]	196.3 ± 9.5 N=8[b]
Relative Maternal Feed Consumption (gd 5 to 6) (g/kg/day)[a]	234.6 ± 35.0 N=9	184.4 ± 7.3 N=8[b]
Relative Maternal Feed Consumption (gd 6 to 7) (g/kg/day)[a]	213.0 ± 8.1 N=8[b]	193.0 ± 5.4 N=9
Relative Maternal Feed Consumption (gd 7 to 8) (g/kg/day)[a]	216.3 ± 19.8 N=7[b]	193.4 ± 7.2 N=9
Relative Maternal Feed Consumption (gd 8 to 9) (g/kg/day)[a]	216.8 ± 15.1 N=9	195.6 ± 5.3 N=9
Relative Maternal Feed Consumption (gd 9 to 10) (g/kg/day)[a]	190.5 ± 7.5 N=8[b]	189.6 ± 5.5 N=9
Relative Maternal Feed Consumption (gd 10 to 12) (g/kg/day)[a]	186.7 ± 8.8 N=9	185.3 ± 5.3 N=9

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 3 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Relative Maternal Feed Consumption (gd 12 to 14) (g/kg/day)[a]	189.2 ± 13.1 N=9	193.6 ± 5.6 N=9
Relative Maternal Feed Consumption (gd 14 to 16) (g/kg/day)[a]	189.5 ± 10.5 N=8[c]	180.2 ± 4.8 N=9
Relative Maternal Feed Consumption (gd 16 to 17) (g/kg/day)[a]	171.5 ± 6.8 N=9	164.0 ± 5.4 N=9
Relative Maternal Feed Consumption (gd 5 to 10) (g/kg/day)[a]	202.6 ± 10.3 N=7[d]	189.8 ± 3.6 N=8[d]
Relative Maternal Feed Consumption (gd 10 to 17) (g/kg/day)[a]	183.7 ± 8.6 N=8[d]	177.9 ± 4.1 N=9
Relative Maternal Feed Consumption (gd 0 to 17) (g/kg/day)[a]	179.4 ± 4.9 N=5[d]	180.1 ± 4.7 N=7[d]

[a]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd = gestational day.

[b]Decrease in N is due to one or more feeders spilling, and therefore an accurate feed weight could not be obtained.

[c]Decrease in N is due to the feed being contaminated with feces and/or urine, and therefore the feed weight was excluded.

[d]Decrease in N is due to interim feed consumption value(s) for one or more dams being missing, and therefore the overall feed consumption value could not be calculated.

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex, and Live Fetal Body Weight (page 1 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
ALL LITTERS[a]	9	9
No. Corpora Lutea per Dam[b]	11.56 ± 1.76 N=9	12.56 ± 1.37 N=9
No. Implantation Sites per Litter[b]	11.78 ± 0.57 N=9	12.33 ± 0.44 N=9
Percent Preimplantation Loss per Litter[b]	9.84 ± 3.84 N=9	8.16 ± 3.05 N=9
No. Resorptions per Litter[b]	3.67 ± 1.41 N=9	1.67 ± 1.30 N=9
Percent Resorptions per Litter[b]	32.72 ±12.92 N=9	13.84 ±10.85 N=9
No. Litters with Resorptions	9	4
% Litters with Resorptions	100.00 **	44.44
No. Late Fetal Deaths per Litter[b]	0.22 ± 0.22 N=9	0.22 ± 0.15 N=9
Percent Late Fetal Deaths per Litter[b]	1.71 ± 1.71 N=9	2.04 ± 1.35 N=9
No. Litters with Late Fetal Deaths	1	2
% Litters with Late Fetal Deaths	11.11	22.22

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex, and Live Fetal Body Weight (page 2 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
No. Nonlive Implants per Litter[b,c]	3.89 ± 1.38 N=9	1.89 ± 1.29 N=9
Percent Nonlive Implants per Litter[b,c]	34.43 ±12.61 N=9	15.88 ±10.70 N=9
No. Litters with Nonlive Implants[c]	9	5
% Litters with Nonlive Implants[c]	100.00 £	55.56
No. Litters with 100% Nonlive Implants[c]	2	1
% Litters with 100% Nonlive Implants[c]	22.22	11.11
No. Adversely Affected Implants per Litter[b,d]	4.00 ± 1.35 N=9	2.00 ± 1.27 N=9
Percent Adversely Affected Implants per Litter[b,d]	35.29 ±12.41 N=9	16.73 ±10.57 N=9
No. Litters with Adversely Affected Implants[d]	9	6
% Litters with Adversely Affected Implants[d]	100.00	66.67
LIVE LITTERS[e]:	7	8
No. Live Fetuses per Litter[b]	10.14 ± 0.74 N=7	11.75 ± 0.65 N=8

Table 5. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex, and Live Fetal Body Weight (page 3 of 3)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
Percent Male Fetuses per Litter[b]		
	52.34 ± 4.33 N=7	44.10 ± 4.72 N=8
No. **Male** Fetuses per Litter[b]		
	5.29 ± 0.52 N=7	5.13 ± 0.55 N=8
No. **Female** Fetuses per Litter[b]		
	4.86 ± 0.51 N=7	6.63 ± 0.75 N=8
Average Fetal Body Weight (g) per Litter[b]		
	0.9697 ±0.0380 N=7	0.9920 ±0.0440 N=8
Average **Male** Fetal Body Weight (g) per Litter[b]		
	0.9875 ±0.0412 N=7	1.0177 ±0.0478 N=8
Average **Female** Fetal Body Weight (g) per Litter[b]		
	0.9424 ±0.0304 N=7	0.9735 ±0.0392 N=8

[a]Includes all dams pregnant at terminal sacrifice on gestational day 20; litter size = no. implantation sites per dam.

[b]Reported as the mean ± S.E.M.

[c]Nonlive = late fetal deaths plus resorptions.

[d]Adversely affected = nonlive plus malformed.

[e]Includes only dams with live fetuses; litter size = no. live fetuses per dam.

£$p<0.05$; Chi-Square Test.

££$p<0.01$; Chi-Square Test.

Table 7. Summary of Morphological Abnormalities in CD-1 Mouse Fetuses: Listing by Defect Type[a]
(page 1 of 1)

	Methyl Tertiary Butyl Ether (mg/m^3, inhaled)	
	0	30000
EXTERNAL MALFORMATIONS		
Total No. of Fetuses Examined for External Malformations[b]	71	94
No. of Fetuses with External Malformations[c]	1	1
% Fetuses with External Malformations	1.4%	1.1%
Total No. of Litters Examined for External Malformations[d]	7	8
No. of Litters with External Malformations[e]	1	1
% Litters with External Malformations	14.3%	12.5%
Cleft Palate	1(1)	1(1)
EXTERNAL VARIATIONS		
Total No. of Fetuses Examined for External Variations[b]	71	94
No. of Fetuses with External Variations[c]	0	0
% Fetuses with External Variations	0.0%	0.0%
Total No. of Litters Examined for External Variations[d]	7	8
No. of Litters with External Variations[e]	0	0
% Litters with External Variations	0.0%	0.0%

[a]A single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).

[b]Only live fetuses were examined.

[c]Fetuses with one or more malformations/variations.

[d]Includes only litters with live fetuses.

[e]Litters with one or more malformed/variant fetuses.

Table A-1. Individual Maternal Body Weights and Organ Weights (g) (page 1 of 1)

		Gestational Day															
Dose[a]	Dam ID	0	5	6	7	8	9	10	12	14	16	17	17[b]	Gravid Uterus Weight	Liver Weight	Paired Adrenal Glands Weight	Paired Kidney Weight
0	1511	24.7	29.0	29.5	30.0	31.5	32.1	33.4	36.2	39.1	44.4	46.1	45.60	11.9625	2.6173	0.0150	0.4708
	1512	27.7	28.3	28.6	29.0	30.0	30.1	30.8	34.3	38.9	44.8	47.5	46.26	15.7460	2.4840	0.0146	0.3892
	1513	27.7	29.2	30.2	30.4	30.4	30.8	32.1	35.2	39.2	45.6	47.9	46.89	15.9233	2.4676	0.0118	0.4999
	1514	25.6	26.2	27.3	27.2	27.7	28.1	28.2	30.7	35.1	40.7	42.8	41.94	12.5632	2.5244	0.0123	0.4241
	1515	26.6	26.6	27.3	27.9	27.5	27.3	27.0	26.9	27.5	28.5	28.2	27.41	0.1131	1.6844	0.0089	0.3874
	1516[c]																
	1517	27.4	29.2	29.6	30.5	30.9	32.5	34.0	37.4	41.9	48.0	50.9	49.88	17.3224	2.4481	0.0126	0.4708
	1518	24.6	23.8	23.7	24.5	25.1	24.8	24.7	24.7	23.9	24.4	24.7	24.34	0.1135	1.3532	0.0085	0.3047
	1519	26.1	26.6	26.7	28.0	27.9	28.4	29.8	33.4	37.7	42.7	45.9	45.26	16.5531	2.3760	0.0101	0.4168
	1520	29.0	31.0	31.6	31.9	33.2	33.4	33.8	37.8	42.4	49.3	52.6	51.39	17.0628	2.6478	0.0109	0.4350
30000	2511	25.3	26.8	26.0	26.2	26.9	27.4	28.7	33.0	38.3	45.1	48.5	48.10	18.8171	2.5123	0.0132	0.4865
	2512	26.8	28.3	27.5	27.9	29.3	29.5	29.8	32.2	37.7	43.3	46.3	45.56	14.9403	2.3680	0.0116	0.3781
	2513[c]																
	2514	26.2	26.7	27.1	27.1	28.2	28.5	29.3	31.7	35.5	42.0	44.9	44.03	13.8178	2.3036	0.0110	0.4367
	2515	26.5	27.3	27.7	28.4	28.5	28.9	29.3	32.7	36.8	43.2	46.1	45.58	17.4442	2.3534	0.0033	0.3982
	2516	27.0	28.3	29.4	29.6	30.4	30.9	32.7	36.9	43.2	50.5	53.0	52.75	21.2817	2.5755	0.0116	0.4759
	2517	29.5	29.3	29.9	30.1	30.3	31.2	31.7	35.5	39.7	48.4	51.3	50.28	19.8939	2.5127	0.0087	0.4627
	2518	25.6	27.5	27.5	27.7	27.8	27.4	27.6	27.7	28.5	28.4	28.2	27.70	0.1236	1.6867	0.0099	0.4376
	2519	27.4	28.5	28.7	29.3	30.1	30.4	31.4	35.6	39.4	46.3	50.0	49.09	18.2453	2.4512	0.0093	0.3942
	2520	27.9	28.6	28.8	29.8	29.5	29.6	30.3	33.9	38.8	43.4	46.9	45.70	15.4457	2.6996	0.0145	0.4027

[a]Mg/m^3 of gasoline MTBE vapor condensate.

[b]Body weight at sacrifice.

[c]Female was not pregnant.

Table A-2. Individual Maternal Feed Consumption (g/day) (page 1 of 1)

Dose[a]	Dam ID	Gestational Days												
		0-5	5-6	6-7	7-8	8-9	9-10	10-12	12-14	14-16	16-17	5-10	10-17	0-17
0	1511	6.0	7.5	7.2	[b]	8.8	5.8	6.9	6.6	9.9	8.7	[c]	7.9	[c]
	1512	4.9	4.9	5.0	5.4	5.1	5.1	5.3	6.5	7.4	7.1	5.1	6.5	5.6
	1513	[b]	15.0	[b]	[b]	7.9	[b]	5.9	8.4	7.9	7.9	[c]	7.4	[c]
	1514	[b]	6.0	5.8	8.4	7.6	6.3	7.3	9.1	8.7	8.1	6.8	8.3	[c]
	1515	4.7	4.7	5.3	4.7	4.4	4.8	4.5	4.6	5.5	5.8	4.8	5.0	4.8
	1516[d]													
	1517	5.8	5.4	6.3	8.1	6.7	6.9	6.1	6.3	[e]	8.0	6.7	[c]	[c]
	1518	5.5	4.3	5.1	4.6	4.2	4.3	4.4	3.7	3.9	4.0	4.5	4.0	4.6
	1519	4.5	5.6	6.1	4.9	5.8	6.2	5.8	6.3	6.4	6.4	5.7	6.2	5.6
	1520	6.3	6.4	7.6	7.5	7.6	6.2	7.3	8.0	8.1	8.2	7.1	7.8	7.2
30000	2511	[b]	4.5	4.8	5.9	6.1	6.1	6.1	8.4	8.0	8.3	5.5	7.6	[c]
	2512	5.2	4.2	5.0	5.7	5.4	4.9	5.3	6.3	6.5	6.7	5.0	6.1	5.5
	2513[d]													
	2514	4.1	5.3	4.6	5.2	5.7	5.1	5.6	6.1	6.6	7.0	5.2	6.2	5.3
	2515	5.1	4.8	5.7	5.2	5.5	5.2	5.1	6.7	6.9	6.6	5.3	6.2	5.6
	2516	6.5	[b]	6.6	6.5	5.3	6.5	6.7	7.5	8.1	8.4	[c]	7.5	[c]
	2517	5.5	6.5	6.0	5.8	6.2	6.1	5.9	7.5	7.9	8.0	6.1	7.2	6.4
	2518	5.9	5.3	5.0	5.8	5.8	5.3	5.8	5.3	5.9	4.7	5.4	5.5	5.6
	2519	6.2	5.1	5.8	5.3	5.9	6.2	6.8	6.9	8.4	7.3	5.7	7.3	6.5
	2520	5.0	5.5	5.7	4.5	5.3	5.3	5.7	7.2	7.4	9.0	5.3	7.1	5.9

[a] Mg/m^3 of gasoline MTBE vapor condensate.

[b] Feed spilled; therefore an accurate feed weight could not be obtained.

[c] Interim feed consumption value(s) are missing and therefore the overall feed consumption value could not be calculated.

[d] Female was not pregnant.

[e] Feed was contaminated; therefore the feed weight was excluded.

Table A-3. Individual Embryo/Fetal Data (page 1 of 5)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect Type	Defect Description
0	1511	11	1	A	L	1	M	1.2080			
			2	E	L						
			3	A	L	2	M	1.1416			
			4	A	L	3	M	1.1652			
			5	E	L						
			6	A	L	4	F	1.1544			
			7	E	L						
			8	A	R	5	M	1.1947			
			9	A	R	6	M	1.2229			
			10	A	R	7	F	0.8638			
	1512	13	1	A	L	1	F	1.0607			
			2	A	L	2	F	0.8947			
			3	A	L	3	F	0.9114			
			4	A	L	4	M	0.6561			
			5	A	L	5	M	0.9103			
			6	A	L	6	M	0.8906			
			7	A	L	7	F	0.9512			
			8	A	R	8	M	0.9243			
			9	A	R	9	M	1.0387			
			10	E	R						
			11	A	R	10	F	0.9565			
			12	A	R	11	M	1.0578			
	1513	18	1	A	L	1	F	0.8099			
			2	E	L						
			3	A	L	2	M	0.9897			
			4	A	L	3	M	0.9108			
			5	A	L	4	F	0.8661			
			6	A	L	5	M	0.8298			
			7	A	R	6	F	0.7425			
			8	F	R			0.2490			
			9	A	R	7	F	0.7755			
			10	A	R	8	F	0.7934			
			11	A	R	9	M	0.8145			
			12	A	R	10	M	0.8377			
			13	A	R	11	M	0.8503			
			14	A	R	12	F	0.8192			
	1514	13	1	A	L	1	F	0.8836			
			2	A	L	2	M	0.9409			
			3	A	L	3	F	0.7931			
			4	A	L	4	M	0.8536			
			5	E	L						
			6	A	L	5	F	0.9164			
			7	A	R	6	F	0.9430			
			8	A	R	7	M	0.9992			
			9	A	R	8	F	0.9298			
	1515	0	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	R						
			6	I	R						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						
			11	I	R						
			12	I	R						
	1516	[g]									

Table A-3. Individual Embryo/Fetal Data (page 2 of 5)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect Type	Defect Description
0	1517	13	1	A	L	1	F	1.0828			
			2	A	L	2	F	1.0061			
			3	A	L	3	M	1.1616			
			4	A	L	4	F	1.0813			
			5	A	R	5	F	0.9930			
			6	A	R	6	M	1.0533	External	Malformation	Cleft Palate
			7	A	R	7	M	0.9497			
			8	A	R	8	M	1.1190			
			9	E	R						
			10	A	R	9	M	0.9991			
			11	A	R	10	M	1.0107			
			12	A	R	11	F	1.0039			
			13	A	R	12	M	1.0375			
	1518	7	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	L						
			6	I	L						
			7	I	L						
			8	I	R						
			9	I	R						
			10	I	R						
	1519	13	1	A	L	1	F	0.8887			
			2	D	L			0.7886			
			3	A	L	2	F	0.8797			
			4	A	L	3	F	0.8849			
			5	E	L						
			6	A	L	4	F	0.9113			
			7	A	L	5	M	0.8804			
			8	D	L			0.7497			
			9	A	R	6	M	0.9570			
			10	A	R	7	F	0.9628			
			11	A	R	8	M	0.9181			
			12	A	R	9	F	0.9405			
			13	A	R	10	M	0.9491			
	1520	16	1	A	L	1	M	0.9749			
			2	A	L	2	M	1.1073			
			3	E	L						
			4	A	L	3	M	1.0296			
			5	A	L	4	M	1.0548			
			6	A	R	5	M	1.1481			
			7	A	R	6	F	0.9627			
			8	A	R	7	F	1.0874			
			9	E	R						
			10	A	R	8	M	0.9004			
			11	A	R	9	F	1.0246			
			12	A	R	10	F	0.8967			
			13	A	R	11	F	0.9971			

Table A-3. Individual Embryo/Fetal Data (page 3 of 5)

Dose[a]	Dam ID#	NCL[b]	Implant #	Type[c]	Position[d]	Fetus #	Sex	Wt.[e]	Defect[f] Exam	Type	Description
30000	2511	16	1	A	L	1	F	0.9097			
			2	A	L	2	F	0.8902			
			3	A	L	3	F	1.0038			
			4	A	L	4	M	1.0807			
			5	A	L	5	M	1.0045			
			6	A	L	6	F	0.9964			
			7	A	R	7	F	1.1017			
			8	A	R	8	F	0.9516			
			9	A	R	9	F	1.0171			
			10	A	R	10	F	0.9881			
			11	A	R	11	F	0.9327			
			12	A	R	12	M	0.9888			
			13	A	R	13	F	0.8993			
	2512	13	1	A	L	1	M	0.8967			
			2	A	L	2	F	0.9912			
			3	A	R	3	F	0.9295			
			4	A	R	4	M	0.9734			
			5	A	R	5	F	0.9657			
			6	E	R	.					
			7	A	R	6	M	0.9817			
			8	A	R	7	F	0.9658			
			9	A	R	8	M	0.8802			
			10	A	R	9	M	1.0423			
			11	A	R	10	F	0.9900			
	2513	.[g]									
	2514	11	1	A	L	1	M	0.9541			
			2	A	L	2	F	0.7654			
			3	A	R	3	M	0.9092			
			4	D	R	.		0.5424			
			5	A	R	4	F	0.8117			
			6	A	R	5	F	0.8227			
			7	A	R	6	F	0.9987			
			8	A	R	7	F	0.9737			
			9	A	R	8	M	0.9013			
			10	A	R	9	F	0.8918			
	2515	17	1	A	L	1	F	0.9424			
			2	A	L	2	M	0.9124			
			3	A	L	3	M	0.8148	External	Malformation	Cleft Palate
			4	A	L	4	F	0.8965			
			5	A	L	5	F	0.8518			
			6	A	L	6	F	0.8904			
			7	A	L	7	F	0.9606			
			8	A	R	8	F	0.9293			
			9	A	R	9	M	0.9842			
			10	A	R	10	M	0.9846			
			11	A	R	11	M	0.9588			
			12	A	R	12	F	0.9191			
			13	A	R	13	F	0.9280			

Table A-3. Individual Embryo/Fetal Data (page 4 of 5)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect Type	Defect Description
30000	2516	14	1	A	L	1	M	1.3223			
			2	L	L						
			3	A	L	2	M	1.2995			
			4	A	L	3	F	1.1934			
			5	A	L	4	M	1.4107			
			6	A	L	5	M	1.3818			
			7	A	R	6	M	1.3251			
			8	A	R	7	F	1.2660			
			9	A	R	8	F	1.2325			
			10	A	R	9	M	1.2527			
			11	A	R	10	F	1.2141			
			12	A	R	11	F	1.2527			
			13	A	R	12	F	1.2403			
			14	A	R	13	M	1.3281			
	2517	15	1	A	L	1	F	0.9643			
			2	A	L	2	F	1.0539			
			3	A	L	3	F	0.9751			
			4	A	L	4	M	1.1039			
			5	A	L	5	M	1.0307			
			6	A	L	6	M	1.0258			
			7	A	L	7	M	1.0202			
			8	A	R	8	F	0.9284			
			9	A	R	9	F	0.9127			
			10	A	R	10	F	0.8877			
			11	A	R	11	F	0.9235			
			12	A	R	12	F	0.9446			
			13	A	R	13	M	1.0347			
			14	A	R	14	F	0.9547			
	2518	3	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	L						
			6	I	L						
			7	I	L						
			8	I	L						
			9	I	R						
			10	I	R						
			11	I	R						
			12	I	R						
	2519	12	1	A	L	1	F	0.9621			
			2	A	L	2	F	0.9309			
			3	A	L	3	M	1.0104			
			4	A	L	4	M	1.0717			
			5	A	L	5	F	0.9861			
			6	A	R	6	M	0.9635			
			7	A	R	7	M	1.0321			
			8	A	R	8	M	0.9550			
			9	A	R	9	F	0.9994			
			10	A	R	10	F	1.0005			
			11	A	R	11	M	1.0335			
			12	A	R	12	M	0.9762			

Table A-3. Individual Embryo/Fetal Data (page 5 of 5)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	2520	12	1	A	L	1	F	0.8464			
			2	A	L	2	M	0.8999			
			3	A	L	3	F	0.9263			
			4	A	L	4	M	0.9872			
			5	A	L	5	F	1.0089			
			6	A	R	6	M	0.9040			
			7	A	R	7	M	0.8767			
			8	D	R	.		0.4609			
			9	A	R	8	M	0.9586			
			10	A	R	9	F	0.8164			
			11	M	R	.	.	.			
			12	A	R	10	M	0.9416			

[a]Mg/m^3 of gasoline MTBE vapor condensate.

[b]Number of corpora lutea.

[c]Implant type codes are as follows: A - Live Fetus; D - Dead Fetus; F - Full Resorption; L - Late Resorption; M - Middle Resorption; E - Early Resorption and I - Implantation Site.

[d]Position refers to uterine horn (R - right, L - left).

[e]Weight is in grams.

[f]Absence of entries under "Exam," "Type," and "Description" for "Defect" indicates no external malformation or variation observed for that fetus.

[g]Female was not pregnant.